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Diversification in the Guiana Shield as seen through frogs

JURY

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Declaration

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Unlike a lot of people, I don't think the *Acknowledgments* is the most interesting part in a PhD dissertation. And it's actually a quite difficult task to accomplish. Most of the time, words do not flow seamlessly through the pen (or keyboard as we most often use in our modern world). For these reasons, at first I wanted to write a very dull *thank you* list, mandatory, nothing more. But then I got caught up in the spiral of writing stuff about people, about experiences, and it's actually quite fun. So here it is, four pages of acknowledgements, I hope it's not too boring nor too long. I also hope that you will find your name in the upcoming lines. If not, I invite you to vigorously express your disappointment and disapproval to the author. Here's my phone number... oh wait, I don't have a telephone.

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Contents

Chapter 1

Introduction

1.1 General context

1.1.1 The Neotropics and Amazonia

The Neotropics constitute one of the major biogeographic realms on the planet (Schultz, 2005). They encompass tropical areas from Central Mexico to Argentina, encompassing different biogeographic area notably: the Carribean region, Amazonia, the Andes, the Chocó (Colombia), the Llanos (Colombia, Venezuela), the Pantepui (Venezuela, Guyana, Brazil), the Caatinga (Brazil), the Chaco (Bolivia) the Cerrado (Brazil), and the Atlantic forest (Antonelli and Sanmartín, 2011; Schultz, 2005). Even though this realm is characterised by a tropical climate, precipitation regimes vary between regions, from almost 9,000 mm on average per year in western Colombia, to almost no precipitations at all in the Atacama Desert in Bolivia. Within the Neotropics, Amazonia is the largest region that covers about 7.5 million km^2 (about 40 % of South America) (Goulding et al., 2003). It is bordered by the Cerrado to the east, the Andes to the west, the Llanos to the north-west. It is characterised by the largest hydrological system on the planet, the Amazon River, that flows along 6400 km from the Andes to the Atlantic. The main tributaries of the Amazon River are, from west to east, the Napo, the Japurá, the Negro Rivers on its left bank, and the Juruá, the Purus, the Madeira, the Tapajós, and the Xingu Rivers on its right bank. The Amazon basin separates two main geological formations, the Brazilian Shield to the south, and the Guiana Shield to the north. It is mostly covered by evergreen tropical forest which covers about 5.5 million km^2, making it the largest rainforest in the

world, but is also peppered with savannahs islands. Precipitations in Amazonia vary from 1,500 to 3,000 mm annually, with an average of 2,000 mm in central Amazonia (Salati and Vose, 1984).

1.1.2 Biodiversity in Amazonia

One of the most spectacular features of biodiversity is the latitudinal gradient in its distribution, with an increase of diversity toward the tropical regions (Dowle et al., 2013; Gaston, 2000; Hillebrand, 2004). Among them, Amazonia is the largest, encompassing about 40 % of tropical forests of the planet, and also the richest, harbouring about 40,000 species of plants, about 2,500 species of terrestrial vertebrates and around 3,000 species of fishes (Antonelli and Sanmartín, 2011; Da Silva et al., 2005; Jenkins et al., 2013; Myers et al., 2000).

Why there are so many species in the tropics, and especially in Amazonia, is a question that has puzzled naturalists since the 19^{th} century (Bates, 1863; de Humboldt, 1820; Wallace, 1852), and still remains debated nowadays (Smith et al., 2014). Answering such a question remains difficult because it involves the interplay of different mechanisms and events that would have promoted speciation over the last 70 million years (Antonelli et al., 2010; Bush, 1994; Hoorn et al., 2010). General principles have been proposed to explain the higher diversity observed in tropical regions, such as the old age of the tropical biotas and higher rates of diversification and lower extinction driven by their climatic stability over long periods of time compared to temperate zones (Moritz et al., 2000; Pyron and Wiens, 2013). Another aspect that have been invoked to explain the high species richness of tropical areas is limited dispersal of species from tropical regions toward temperate ones, and conversely more dispersal of temperate species toward tropical regions dispersal (Pennington and Dick, 2004; Pyron and Wiens, 2013). Several hypotheses linked to geomorphological (uplift of the Andes, marine incursions), hydrological (formation of large river drainages), climatic (fragmentation of landscape triggered by cooler and dryer climates) events have been formulated to explain the build-up of the Amazonian biodiversity. The recent acquisition of geomorphological, hydrological and palaeoclimate data in Amazonia and adjacent regions, as well as diversification patterns retrieved from molecular analyses, enabled to test the concomitance of historical events and cladogenesis, and

therefore discuss the relevance of the different hypotheses on the origin of diversification in Amazonia. Paradoxically, even though Amazonia is the richest region on earth, it is also one of the less studied region in terms of phylogeography (Beheregaray, 2008), resulting in many gaps in our knowledge on diversification patterns, species limits, species ranges. Therefore, data acquisition on different groups throughout Amazonia is needed in order to formulate strong hypothesis for explaining the origin of Amazonian diversity .

1.2 Hypotheses for the origin of Amazonian diversity

The origin of the vast biodiversity observed nowadays in Amazonia has been highly debated, notably whether it mostly stemmed from diversification triggered by Neogene geomorphological events or by recent Quaternary climatic oscillations (Hoorn et al., 2010; Moritz et al., 2000; Rull, 2008, 2011). Actually, most diversification events in Amazonian lowlands happened in the last 20 million years (Hoorn et al., 2010). Molecular phylogenies of different groups show that both Neogene geomorphological events as well as Quaternary climatic oscillations have played a role in Neotropical diversification (Rull, 2011). The orogenesis of the central Andes that began in the early Miocene was a process that triggered high rates of speciation in this region. A global cooling of temperatures that occurred throughout the Miocene and the Pliocene would have favoured dispersal events from the Andes to the neighbouring lowlands of Amazonia. Speciation within Amazonia certainly resulted from different processes linked with the setting-up of the Amazonian drainage about 10 mya, the formation of large rivers of the Amazon drainage, and by formation of disconnected refugia. For example, it has been inferred that *Charis* butterflies diversified during the Miocene, through vicariance that occurred with the setting-up of major Amazonian rivers (Hall and Harvey, 2002). On the contrary, other organisms such as *Saimiri* squirrel monkeys or *Psophia* trumpeters birds have diversified more recently, in the late Pliocene and during the Pleistocene (Lavergne et al., 2010; Ribas et al., 2012). It has been hypothesised that diversification within *Saimiri* was influenced by loss of connection between forest refugia induced by climatic oscillations during this period (Lavergne et al., 2010), whereas river dynamic has been invoked as a driver to speciation by successive dispersals in *Psophia* (Ribas et al., 2012).

Four main hypotheses (Andean uplift, marine incursions, riverine barriers, and forma-

tion of refugia) have been formulated to explain the origin of the enormous diversity that is observed nowadays in Amazonia, evoking events that happened from the Neogene to the Quaternary.

1.2.1 Miocene marine incursions and Andean uplift in the early Miocene

Repeated marine incursions in northern South America occurred since the late Eocene (∼40 mya), and culminated in the early Miocene (23–15 mya) with the Pebas formation in the western part of northern South America, that consisted of a large lakes and drainage that ran from south to north (Fig. 1.1) (Hoorn, 1993; Hoorn et al., 2010). It has been hypothesised that such large and long lasting wetland formations had an impact on terrestrial organisms in fragmenting the landscape, thus leading to loss of connection between populations over a long period of time (Lovejoy et al., 1998; Nores, 1999). It is for example coincidental with the split between the two Neotropical frog families Allophrynidae and Centrolenidae in the late Eocene and could have caused the isolation of the proto-Andes from the rest of Amazonia, thus triggering the diversification event in between both clades (Castroviejo-Fisher et al., 2014). Similarly, the role of marine incursion has been evoked to explain diversification within the Neotropical snake genus *Corallus* (Colston et al., 2013), as well as for Dendrobatid frogs (Noonan and Wray, 2006) for example. The orogeny of central Andes began at the same period, around 20 mya. Several studies showed that this geological event certainly played a major role in species diversification during the Neogene, and the Andes are considered to be the centre of origin of many Neotropical clades that diversified mainly through vicariance of populations that occurred on either sides of emerging mountains, or montane taxa separated by deep valleys or impassable peaks (Antonelli et al., 2010; Antonelli and Sanmartín, 2011). It has been suggested that subsequent dispersal of plants and animals from the Andes towards adjacent zones in Amazonia was triggered by a cooling of temperatures that began during the mid-Miocene (∼15 mya), but became more important and rapidly fluctuating in the last 3 my until the Quaternary (Antonelli et al., 2009; Castroviejo-Fisher et al., 2014; Elias et al., 2009; Haffer, 1997; Hughes and Eastwood, 2006; Lynch and Duellman, 1997; Santos et al., 2009). However, many diversified clades in Amazonia do not occur in the Andes (e.g., Leptodactylidae, Microhylidae, Phyllomedusidae, Lophyohylinae, *Amazophrynella*,

Scinax). Therefore, their diversification cannot be directly linked to the orogeny of the Andes via dispersal from higher elevation localities toward Amazonia.

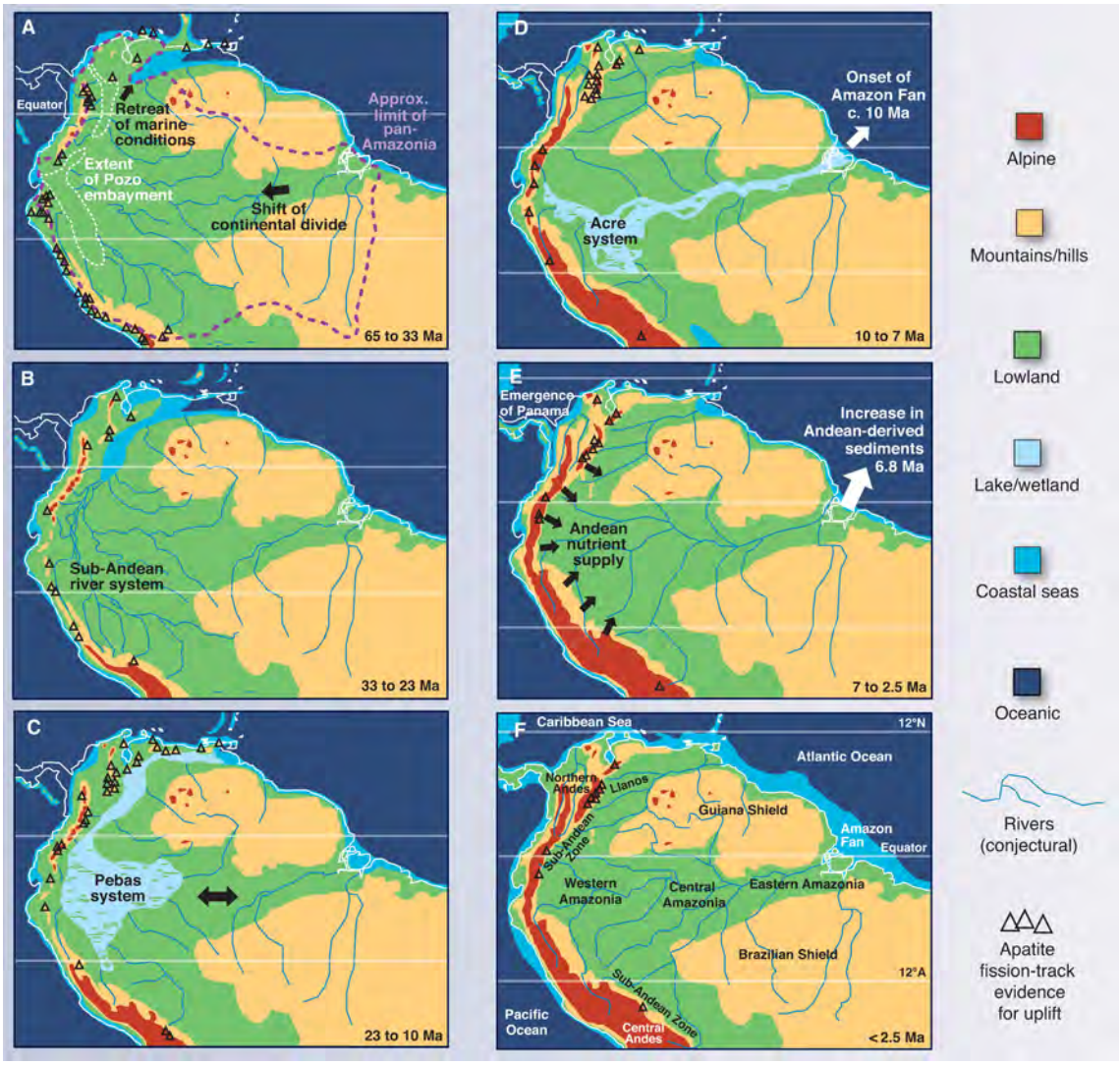

Figure 1.1 – Evolution of the Amazonian landscape through time, from Paleogene to Quaternary periods. (A) Paleocene and Eocene; (B) Oligocene; (C) early Miocene to late Miocene; (D) late Miocene; (E) late Miocene to early Pleistocene (F) Pleistocene and Holocene. Figure taken from Hoorn *et al.* 2010.

1.2.2 Setting-up of the Amazon drainage in the late Miocene

The uplift of the central and northern Andes from in the Miocene had a profound impact on the landscapes of northern South America, as it resulted in the retreat of the Pebas system, and the formation of the Amazon basin during the late Miocene (10–7 mya) that ran nowadays from west to east (Fig. 1.1).

Naturalists who travelled in Amazonia in the 19^{th} century observed allopatric distribution of species on opposite banks of three major rivers, the Amazon, the Madeira, and

the Negro rivers (Wallace, 1852; Hellmayr, 1910). It has been hypothesised that the formation of major Amazonian rivers during the Pliocene might have generated allopatric speciation by limiting or preventing dispersion between contiguous regions separated by large rivers through a riverine barrier effect (Antonelli et al., 2010). In such cases, divergence between sister species should be reflected in their allopatric range along rivers, as well as the setting-up of the Amazon drainage during the late Miocene. Several examples support this hypothesis, like the butterfly *Charis cleonus* species complex (Hall and Harvey, 2002), as well as in primate genera (*Cebus*, *Callicebus*, and *Cacajao*) (Boubli et al., 2015), and in the bird *Myrmeciza hemimelaena* species complex (Fernandes et al., 2012). Other evidences of the riverine barrier effect on bird diversification have been documented in Amazonia, for example in the *Hypocnemis cantator* clade (Tobias et al., 2008), in *Pteroglossus* (Patel et al., 2011).

However, it has been argued that in some cases it is difficult to interpret if the river was a location of primary diversification, or if it stands as a meeting point for species that diverged elsewhere and then dispersed up to the river (Bates et al., 2004; Funk et al., 2007b; Moritz et al., 2000). Also, some studies conducted on butterflies, frogs, and small mammals failed at finding a barrier effect of some Amazonian rivers such as the Juruá River in western Amazonia (Elias et al., 2009; Gascon et al., 2000; Patton et al., 1994, 2000). The physical characteristics of some rivers might actually explain such patterns, as it has been hypothesised that large meandering rivers might be permeable to gene flow through passive transfer triggered by river dynamics. In such cases, gene flow would be possible through the formation of islands that can be connected to either one or the other banks through time (Bates et al., 2004; Peres et al., 1996). On the contrary, fast-flowing rivers that have a stable course over long time scales such as the Madeira or the Tapajós rivers would have acted as barriers to gene flow and might have promoted allopatric speciation (Bates et al., 2004). Such a pattern has been found for example in *Psophia* along the Madeira and Tapajós rivers (Ribas et al., 2012).

1.2.3 Climate cycles in the Pliocene and Pleistocene: The refugia hypothesis

This hypothesis has been formulated in the late 1960's by German ornithologist Jürgen Haffer (Haffer, 1969). In a first article published in 1969, Haffer stipulated that climatic oscillations during the late Pliocene and Pleistocene induced fragmentation of the rainforest during cycles of cooler and dryer climates, resulting in a landscape of disconnected forest patches scattered across interfluves. Populations were thereby isolated for long periods of time, thus leading to genetic divergence, and eventually to speciation.

Even though this hypothesis has been criticised given molecular studies showed that most diversification events in Amazonia largely predate the Pleistocene (Antonelli et al., 2010; Bush, 1994; Hoorn et al., 2010), recently published data provided evidence of Pleistocene diversification within Amazonia in mammals and birds (Lavergne et al., 2010; Patel et al., 2011; Ribas et al., 2011, 2012). Quaternary glacial cycles certainly had major impacts on Neotropical landscapes, as cooler and dryer climates during these glacial periods may have had a major impact on Neotropical forest either through forest fragmentation, forest composition, or species specific ranges modifications (Carnaval et al., 2009; Mayle and Power, 2008). Also, even though the putative formation of refugia during the Pleistocene may not be the primary cause of most speciation events in Amazonia, it may have helped maintaining the genetic structure that is observed nowadays as rivers between refuges served as meeting points for species that diverged elsewhere (Haffer, 1997).

1.2.4 In summary

Several evidences indicate that most of Amazonian biodiversity resulted from divergence events that occurred in the last 20 million years (Hoorn et al., 2010), and that it originates from both *ex situ* processes that happened in neighbouring regions such as the Andes, and *in situ* processes such as formation of large rivers and formation of refugia during the Neogene and Quaternary. Actually, these hypotheses are not exclusive, and it is highly probable that an interplay of different factors actually helped shape the diversity currently observed in Amazonia (Aleixo, 2004; Bush, 1994; Rull, 2011). One of the main reasons why it is difficult to come up with a robust corpus of hypotheses that could be tested to explain the origin of Amazonian diversity lies in the simple fact that diversity

in Amazonia still remains vastly unknown and many species remain to be discovered or described (Bush and Lovejoy, 2007; Hopkins, 2007). Also, the data on the distribution of species is largely inaccurate, simply due to the fact that most areas of Amazonia have not been explored, or that species are not well-defined, thus leading to an under- or overestimation of species ranges. Such inaccuracies in our knowledge of species delineation and distribution, respectively named Linnean and Wallacean shortfalls (Lomolino and Heaney, 2004), hamper large-scale studies on diversity in Amazonia.

1.3 The Linnean and Wallacean shortfalls

The inventory of diversity on earth is far from being complete, and documenting all the species that are currently living on the planet remains a colossal task. Still, estimations of the total number of species of plant and animals on the planet have been attempted. These estimates vary a lot according to sources, from 3 to 100 million species (May, 2010), and even up to one trillion when considering microbial species (Locey and Lennon, 2016), and only about one million species have been described (Mora et al., 2011). Also, if distribution of species is rather well-known in temperate countries, at least for some groups (*e.g.*, terrestrial vertebrates, Odonata, Lepidoptera), the situation is very different for tropical regions, where distribution are aften incomplete, when not completely lacking (Lomolino and Heaney, 2004; Bini et al., 2006). Describing all species that inhabit earth is a goal that will certainly never be reached, even though such a feat remains important for conservation purposes (Mora et al., 2011). Therefore, it would be necessary to have good estimates for some key groups that could then be used as models. Groups such as amphibians would be good candidates as they are not too diverse (compared with wingless arthropoda for example), they do not disperse a lot compared to birds for example, and they are sensitive to variation in abiotic conditions. Getting a better resolution in species delineation and species distribution in key groups is needed to enhance our knowledge on the evolutionary history of species and on the processes of diversification. Such a requirement is actually strongly needed in Amazonia as it is a vast and megadiverse region in which a large part of the diversity remains to be described and for which biogeographical and evolutionary histories of species and of assemblages are still not well understood. In other words, the understanding of the basic structure of diversity is a prerequisite to be

able to investigate the processes of diversification, as well as conservation biology.

1.4 Bioregions

Biogeographical regions (hereafter referenced to as "bioregions") are defined as 'geographically distinct assemblages of species and communities' (Vilhena and Antonelli, 2015). Defining bioregions is a key component in evolutionary biology for understanding the historical processes that helped shape diversity and the distribution of species (Harold and Mooi, 1994; Vilhena and Antonelli, 2015). Bioregionalisation dates back from the 19^{th} century with the works of Alfred Russell Wallace (Wallace, 1876), who divided the whole planet into six zoogeographical regions (Fig. 1.2).

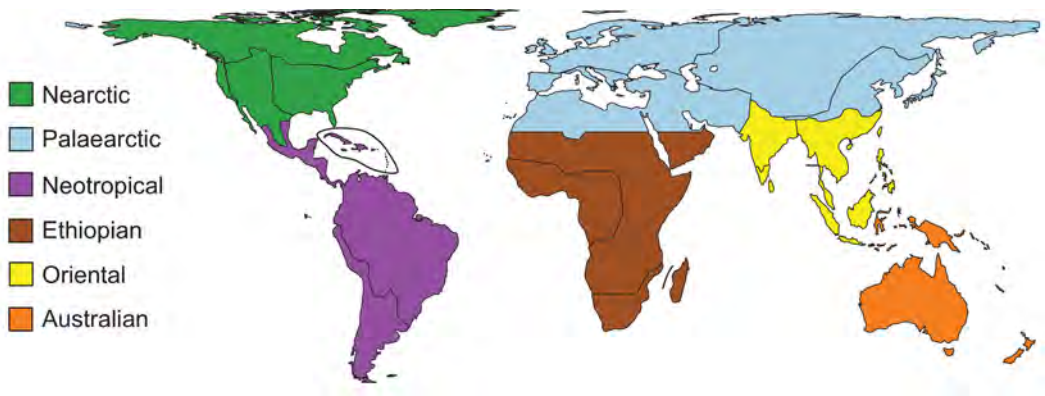

Figure 1.2 – The six zoogeographical regions of the world as defined by Alfred Russel Wallace in his book *The Geographical Distribution of Animals* published in 1876. Map taken from Kreft and Jetz (2010).

Since the classification of Wallace, other attempts of bioregionalisation of the planet have been made, notably by Smith (1983) who defined ten bioregions based on the occurrence of mammal families. More recently, bioregionalisation at the scale of the planet was carried out using large databases of distribution of terrestrial vertebrates (Holt et al., 2013; Kreft and Jetz, 2010), that permitted to redefine some boundaries (for the Paleartic realm for example in Holt et al. (2013)), and to add new realms (Panamanian, Sino-Japanese, and Oceanian realms in Holt et al. (2013)). Bioregionalisation has also been attempted at more local scales, for example in North America with mammals (Escalante et al., 2013), in the Brazilian Atlantic Forest with amphibians and snakes (Moura et al., 2016; Vasconcelos et al., 2014), as well as in Australia (Ebach et al., 2013). These studies used various methods to characterise bioregions, and recently, various methodological frameworks to

conduct bioregionalisation based on species distribution have been proposed (Kreft and Jetz, 2010; Vilhena and Antonelli, 2015).

Bioregionalisation within Amazonia also dates back from the 19^{th} century with the works of Wallace. He was the first to report that species compositions between different regions of Amazonia were not similar, and that large rivers might act as barriers between regions with different species assemblages (Wallace, 1852). He thus defined four 'districts': Guiana, Peru, Ecuador, and Brazil districts, whose boundaries were determined by three main rivers, the Amazon, the Negro, and the Madeira (Wallace, 1852). Since Wallace's works, an updated bioregionalisation of Amazonia was attempted by examining the distribution boundaries of endemic birds, and nine Amazonian 'areas of endemism' were thus identified (Borges, 2007; Cracraft, 1985; da Silva et al., 2002). One of these is the Guiana area of endemism, that encompasses the lowlands of the Guiana Shield and is bounded south by the Amazon River, west by the Rio Branco, and north by the Pantepui and the Orinoco delta. Even though there was some other attempts to characterise bioregions in the Neotropics (Vasconcelos et al., 2011), to our knowledge, no study explored bioregionalisation at the scale Amazonia using occurrences of large communities such as what was done at a global scale using vertebrates. More recently, a study conducted on avian distribution and endemism refined the definition of boundaries of the Guiana region, and actually showed that the Rio Branco and the associated savannahs constitute important boundaries (Naka, 2011). The implication of these findings is that the actual western boundary of the Guiana area of endemism is not the Rio Branco, but rather the Negro–Branco interfluvium, which constitutes a transition zone between an avifauna distributed east of the Rio Branco and the other west of the Rio Negro (Naka, 2011).

1.5 The Guiana Shield and its diversity

The Guiana Shield is a vast geologic entity in northeastern South-America, ranging from about 50° W to 74° W of longitude and 3° S to 9° N of latitude (Fig. 1.3). It is delimited by the Orinoco River to the north and by the Amazon River to the south-east and the Japurá to the south-west. Three main regions can be distinguished within the Guiana Shield: (1) the Pantepui region to the west, characterised by highland habitats, (2) the western lowlands in Colombia, and (3) the Amazonian lowlands of the eastern Guiana Shield

(EGS). The Pantepui region covers about 48,700 km^2, and is formed by tabular mountains composed of Precambrian sandstones (the *tepuis*) occurring in Venezuela, Guyana, and northern Brazil, and which altitudes range between 1,000 and 3,000 m a.s.l. (Lujan and Armbruster, 2011; Mayr and Phelps, 1967). These highlands are faunistically very distinct from the rest of the continent with many endemic lineages (Berry and Riina, 2005; Désamoré et al., 2010; Kok et al., 2012, 2016a,b; Rull, 2004, 2005; Salerno et al., 2012). On the contrary, the EGS is faunistically part of Amazonia and is mainly constituted by lowland forests. It encompasses the states of Pará, Roraima, Amazonas, and Amapá (Brazil), French Guiana, Guyana, and Suriname.

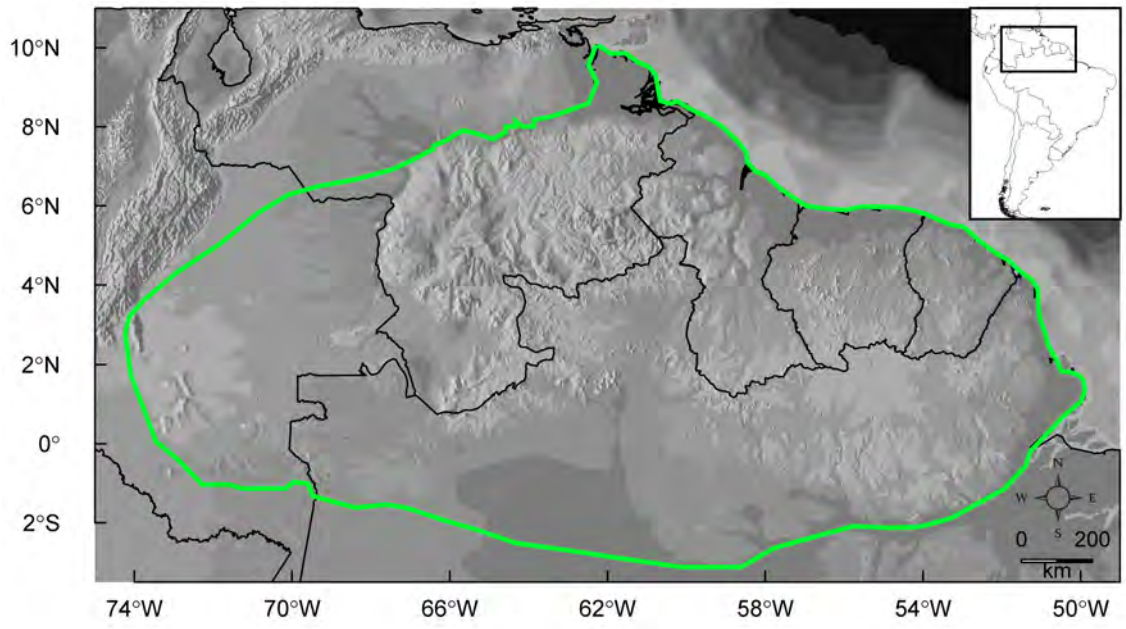

Figure 1.3 – Map of northeastern South America showing one of the commonly admitted limits of the Guiana Shield (green line).

The Guiana Shield is a very diverse region, with about 3,000 species of vertebrates and 13,367 vascular plants (Funk et al., 2007a; Hollowell and Reynolds, 2005; Vari and Ferraris, 2009). Several endemic species characterise the assemblages within the Guiana Shield: vascular plants (43 % of endemic species), birds (7.7 % of endemic species), mammals (11 % of endemic species), reptiles (29 % of endemic species), amphibians (54 % of endemic species) (Hollowell and Reynolds, 2005). Many of these endemics are actually restricted to the high elevations habitats of the Pantepui region (Huber, 2005; Berry and Riina, 2005; Désamoré et al., 2010; Kok et al., 2016a; Leite et al., 2015; Salerno et al., 2012).

1.6 Diversity of amphibians in the Guiana Shield

Northern South America is the most diverse region for amphibians worldwide (Pyron and Wiens, 2013). The amphibian fauna is particularly rich in the Andean region, and in western Amazonia (Duellman, 1999). In the Guiana Shield (lowlands and highlands altogether), 263 species were known in the mid-2000's, of which 253 were anurans, and with 54 % of endemic species (Señaris and MacCulloch, 2005). Most endemic species are only found in the *tepuis* (e.g. *Ceutomanthis*, *Myersiohyla*, *Oreophrynella*, *Stefania*, *Tepuihyla*), and few endemic groups are distributed throughout the Guiana Shield such as the Guianan clade of *Adelophryne*, and the genera *Anomaloglossus*, *Otophryne*, and *Synapturanus*. The other frog lineages occurring in the EGS are nested within clades diversified throughout Amazonia (Castroviejo-Fisher et al., 2014; Fouquet et al., 2007b, 2012b, 2015b, 2016; Peloso et al., 2014; de Sá et al., 2014). Such a pattern indicates both dispersal between the EGS and Amazonia, and diversification within the Guiana Shield. Since 2005, several species have been described from the *tepuis* (Barrio-Amorós et al., 2010; Barrio-Amorós, 2010; Kok et al., 2006b, 2010; Kok, 2013) as well as from the eastern lowlands (Castroviejo-Fisher et al., 2011; Fouquet et al., 2007a, 2015a,b, 2016; Kok et al., 2006a; Peloso et al., 2014). Moreover, several recent molecular-based studies suggested that the current diversity of frogs in the Guiana Shield (as well as in Amazonia in general) is vastly underestimated (Fouquet et al., 2007c,b; Funk et al., 2012; Kok et al., 2012).

1.7 Goals of this PhD

Identifying the processes that are responsible for the remarkable Amazonian diversity remains challenging because species limits are not well-resolved and their distributions are poorly known. During the course of my PhD project, I tackled to tackle such a vast issue by focusing on one particular region, the Guiana Shield lowlands, and using anuran amphibians as models. My dissertation is divided into three parts: (1) refining species delineation and distribution in the eastern Guiana Shield (EGS) and propose a bioregionalisation in this area, (2) explore species delineation within the genus *Anomaloglossus*, and (3) study the biogeographic history of this genus.

The first aim was to test if the Eastern Guiana Shield as defined by Naka (2011) based on the distribution of endemic birds represents an actual and relevant bioregion for anurans. In order to achieve this goal, I estimated the number of anurans and their distribution in the EGS, and explored bioregionalisation. Because of the importance of the Linnean and Wallacean shortfalls in Amazonia, the first step was to collect as much mtDNA data as possible on as much species of frogs as possible throughout the EGS in order to provide a relevant input for the biogeographic analysis at a large scale.

Within the Guiana Shield, one frog genus, *Anomaloglossus*, is remarkable being the only endemic group that has diversified importantly (26 species), both in the Pantepui region and in the lowland forests of the EGS. What is even more striking is the diversity of reproductive strategies found within this endemic genus, as four modes have been documented until now: (1) phoresy and exotrophy (Grant et al., 2006); (2) phoresy and endotrophy (Lescure, 1975); (3) maternal care in phytotelms and exotrophy (Kok et al., 2006a,b, 2013); (4) nidicoly and endotrophy (Junca et al., 1994). Because of these aforementioned characteristics, *Anomaloglossus* constitutes a very interesting model to study diversification patterns and processes of diversification in the Guiana Shield. Delineating species within this genus is challenging as it certainly harbours cryptic species (Fouquet et al., 2007b, 2012a; Kok et al., 2012). The second aim of this PhD was to address the question of species delineation by using an integrative approach, combining molecular, morphological, bioacoustical, and data related to life-history traits.

The third and final aim of this PhD was to explore the spatio-temporal aspects of the diversification of *Anomaloglossus* within the Guiana Shield. The questions addressed in this chapter were to determine the centre of origin of *Anomaloglossus*, as well as testing if the acquisition of different reproductive modes was linked to major diversification events. I applied a phylogenetic approach generating data that would enable to yield a well-resolved time-calibrated phylogeny (New Genereation Sequencing to produce mitogenome sequences and multiple unlinked nuclear loci) to infer the evolutionary relationships within the focal group, and also to explore the historical pattern of diversification.

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Chapter 2

Barcoding Amazonian amphibians reveals vast underestimation of species richness and allows estimating biogeographical boundaries and endemism in the Guiana Shield

Foreword to Chapter 2

Biogeographical analyses of this chapter were conducted in collaboration with Guilhem Sommeria-Klein (EDB-ENS) and Francesco Ficetola (CNRS-LECA).

2.1 Introduction

Amazonia encompasses about 40% of tropical forests of the world (Hoorn and Wesselingh, 2010; Hubbell et al., 2008), and hosts the highest species richness on earth for many taxonomic groups (Antonelli and Sanmartín, 2011; Jenkins et al., 2013). The processes that have shaped this diversity have long intrigued biologists (Bates, 1863; Wallace, 1852), especially due to the apparent homogeneity of the vast uniform extent of forest. However,

the apparent homogeneity within this region is misleading as landscape, temperatures and rainfall vary widely (Mayle and Power, 2008) as well as vegetation types (Anderson, 2012; Hughes et al., 2013). Moreover, Amazonia had a tumultuous climatological and geological past, mainly caused by the Andean uplift and the setting-up of the Amazon River watershed during the late Tertiary (Hoorn et al., 2010).

The distribution of species within Amazonia relates to this large-scale environmental heterogeneity, and in groups such as forest birds and primates, congruence between geographic distribution patterns and major interfluves led to the definition of biogeographic subregions (BSR) that were coined as "Amazonian areas of endemism" (Cracraft, 1985; Haffer, 1974; Wallace, 1852). However, there is little agreement on how to best classify, delimit, and name BSR, with many terms being used, often interchangeably (Vilhena and Antonelli, 2015). In fact, the very existence of different BSR across Amazonia, as well as the relative degree of endemism within them, have been largely understudied, with very few unambiguous distribution data on large faunal assemblages using modern analytic tools (e.g., Nelson et al. (1990); Morrone (2005) but see Naka et al. (2012)). Current knowledge on the delimitation of Amazonian BSR is mostly based on birds, which certainly constitute the best-known taxonomic group in Amazonia. Yet, accepted limits of Amazonian BSR vary substantially among bird groups (Da Silva et al., 2005; Morrone, 2005; Ribas et al., 2009) and the good dispersal abilities of birds can determine a lower biogeographical structure compared to other taxa (Claramunt et al., 2011; Pigot and Tobias, 2015). Therefore, the explanatory power of the Amazonian BSR remains limited until their boundaries is proven to match across groups. Using assemblages of small terrestrial vertebrates such as amphibians to delineate bioregions might produce different/finer patterns, notably because of their limited dispersal abilities and their sensitivity to environmental variations (Zeisset and Beebee, 2008). As a matter of fact, Amphibians have proven to represent an ideal group for such an approach both at the continental (Vilhena and Antonelli, 2015) and regional scales (Vasconcelos et al., 2014). One of the rare studies unambiguously delimiting BSRs in Amazonia showed a strong correlation between the distribution limits of birds across the Rio Negro and the Rio Branco but a relative homogeneity within the Eastern Guiana Shield (EGS) (Naka et al., 2012). However, other studies on amphibians revealed a finer pattern with concordant distribution limits of divergent lineages of at least 11 frog species within this region, and suggest a

higher rate of endemism than what is currently admitted (Fouquet et al., 2012c, 2013, 2016).

Defining the basic geographical structure of the diversity within Amazonia is an important prerequisite for the study of the processes that gave rise to the current diversity. Many hypotheses have been formulated to explain allopatric patterns of distribution across Amazonia, involving landscape change induced by late Tertiary climate change (Haffer, 1969), the uplift of the Andes and continuous dispersal across large rivers (e.g., Hayes and Sewlal 2004; Antonelli et al. 2010; Hoorn et al. 2010), or past environmental gradients (e.g., Colinvaux et al. 2000). These different hypotheses have been verified for some taxonomical groups at different spatial and temporal scales (e.g., Hall and Harvey 2002; Brumfield et al. 2007). However, there is still no consensus about the main drivers of diversification within Amazonia partly because species boundaries and the basic structure of the Amazonian biodiversity remain very imprecise. Identifying BSRs in Amazonia will help to detect the relevant barriers involved in the speciation processes, secondary contacts, and dispersal limitations ultimately allowing formulation of strong hypotheses that could then be tested with complementary data. Defining biogeographic regions within Amazonia is also of crucial importance for conservation given the unique diversity of Amazonia is under strong pressure from human disturbance, mainly via habitat loss and climate change (Cox et al., 2004; Soares-Filho et al., 2006; ter Steege et al., 2015). Bioregionalisation of Amazonia would enable to estimate regional rates of endemism and identify areas of high priority for implementing conservation actions at a large scale, such as the definition of conservation corridors, or the delimitation of protected areas (Da Silva et al., 2005; Young et al., 2009).

One of the main challenges to improve our understanding of species boundaries and distribution lies in the scarcity of occurrence data and the imprecision of species delineation (Wallacean and Linnean shortfalls) in Amazonia. These shortfalls are particularly obvious in most terrestrial organisms such as amphibians. Almost all amphibian species supposed to have broad ranges in Amazonia that have been studied turned out to harbour deep divergences when analysed with genetic tools, suggesting that they are actually composed by several species each with restricted distributions (Fouquet et al., 2007b, 2015b, 2016; Funk et al., 2012; Gehara et al., 2014; Ferrão et al., 2016). These studies typically

estimate that the actual species richness was at least twice what is estimated from morphology only. Therefore, distribution ranges of Amazonian amphibians obtained from broad biodiversity assessments such as the IUCN Red list are likely to be largely inaccurate (Ficetola et al., 2014). According to IUCN, 427 amphibian species inhabit the 5.5 to 6 million km^2 of Amazonia, with at least 150 species (35%) with broad distribution ($>$ 1 million km^2) (Fouquet et al., 2007a). However, as amphibians display low dispersal capacities and often have small niches (Duellman and Trueb, 1994; Wells, 2010), such a proportion is rather unlikely (Wynn and Heyer, 2001). This gap in our understanding of the actual diversity and the distribution of the species could have consequences on the many analyses made from IUCN data (Foden et al., 2013; Jenkins et al., 2013, 2015; Pimm et al., 2014; Feeley and Silman, 2016).

The overall aims of this study were (1) to obtain a new georeferenced dataset of Amazonian anurans based on molecular diversity, with a focus on the eastern Guiana Shield (EGS) (east of the *tepuis*, and north of Amazon and Negro rivers), (2) to provide estimates of the number of species and their distributions in this part of Amazonia, (3) search for the spatial boundaries among BSRs as well as re-assess the rate of endemism within this area. Given that amphibian species boundaries and distributions are plagued with uncertainty in Amazonia and that IUCN data are often out-dated and imprecise, it is necessary to use occurrence records linked to taxonomic frameworks based on clear criteria. Therefore, we collected an unprecedented DNA barcode database (16S rDNA), representing the largest dataset of molecular diversity in Amazonia gathered so far, in order to assess the actual number of anuran species, the spatial boundaries of BSR of the EGS, as well as the rate of endemism within this area.

2.2 Material and methods

2.2.1 Field work

We undertook fieldwork in several localities throughout the Guiana Shield, notably in southern Suriname, French Guiana, and the Brazilian states of Amapá and Roraima. We collected specimens of as many anuran species as possible per locality by nocturnal and diurnal active searches (visual and acoustic) and with pitfall traps. Specimens were

identified and photographed, before being euthanized using an injection of Xylocaine® (lidocaine chlorhydrate). Tissue samples (liver or muscle tissue from thigh or toe-clip) were removed and stored in 95 % ethanol, while specimens were tagged and fixed (using formalin 10 %) before being transferred to 70 % ethanol for permanent storage. These field surveys allowed us to cover the anuran communities of the EGS at an unprecedented fine scale (Fig. 2.1). We completed these data with loans of material, notably from the upper Madeira, lower Xingu, Abacaxis and Purus Rivers, allowing delimiting putative BSR boundaries. Ultimately, the total number of newly analysed samples reached 4,681.

2.2.2 Molecular data

We extracted DNA from the 4,681 samples using the Wizard Genomic extraction protocol (Promega; Madison, WI, USA). We targeted a c.a. 400bp fragment of the 16S rDNA. We used primers N16R and N16F (Salducci et al., 2005), to which we added NNN + 8-nucleotides labels (hereafter designated as 'tags') for sample identification as all resulting PCR products were mixed into libraries: 32 tags for forward primer (N16R) and 36 tags for reverse primer (N16F). PCRs were carried out in a final volume of 20 μl, and contained 2 μl of 50ng/μl DNA extract, 10μl of AmpliTaq Gold® 360 Master Mix (Life Technologies, Carlsbad, CA, USA), 5.84 μl of Nuclease-Free Ambion Water (Thermo Fisher Scientific, Massachusetts, USA), 0.25 μM of each primer and 3.2 μg of bovine serum albumin (BSA, Roche Diagnostic, Basel, Switzerland). The PCR conditions were as follow: 95°C for 10 min, then 40 cycles of 95°C for 30 s, 46°C for 30 s, 72°C for 30 s, followed by a final step of 72°C for 7 min. We prepared three complete libraries, each containing 1152 samples, including 72 blanks (6 blanks per plate). Libraries of mixed PCR products were sequenced using 2 250 paired-end sequencing technology through MiSeq high throughput sequencing (Illumina) at the Génopole (Toulouse, France). We generated 4,492 new sequences, among which 3,148 were retrieved from MiSeq and 1,344 were retrieved from Sanger sequencing.

Additionally, we retrieved all sequences of congeneric species occurring in the Guiana Shield from GenBank (stopped on 1st August 2015), as well as sequences of *Adelphobates* and *Phyzelaphryne*, two genera restricted to southern Amazonia (n = 6673). We examined these sequences, and low quality and short sequences were removed as well as duplicates.

We obtained approximate geographical coordinates for most of these records searching the original papers, locality information, or collection databases. The final dataset contained 11,166 terminals, 10,254 of which were geotagged. This barcode dataset is probably the most extensive in this region gathered so far for any vertebrate group: 8181 records are from Amazonia including 4634 from the EGS. The obtained sequences were aligned with MAFFT v.7 (Katoh and Standley, 2013). We used the resulting alignment to generate a neighbourg-joining tree using pairwise deletion and p-distance model with MEGA v.7.0.16 (Kumar et al., 2016).

2.2.3 Taxonomic framework

We used two different taxonomic frameworks that we applied to our sequence dataset. First, we generated a conservative framework (TAXO1) based on the neighbour-joining tree that we generated using all 16S sequences. Species identifications were in many cases modified from the original fieldwork and GenBank assignments because many sequences were unidentified to the species level (sp.), clearly misidentified or because taxonomic changes occurred subsequently to sequences submission. In these cases, we assigned these sequences to the closest nominal taxon based on genetic affinities, known range and the location of the type locality of each taxon as indications. Many species remained also polyphyletic, including some already pointed out as species complexes (e.g., *Dendropsophus minutus*). We kept conservative identification when they formed monophyletic groups. When paraphyly was ambiguous, we kept the original identification. Despite this conservation rationale, many terminals could not be assigned to any nominal taxon, and therefore we used "sp.". In some opposite cases, two or more taxa were largely intricate with shallow genetic distances among terminals and remained ambiguous even given the distribution of the lineages. We then considered them as single taxon (e.g. *Atelopus hoogmoedi*, *A. flavescens*). Ultimately, we think that our taxonomic framework reached a conservative update of the current taxonomic knowledge for Amazonian anurans.

Secondly, we produced a less conservative framework (TAXO2) by applying the Automatic Barcode Gap Discovery (ABGD) species delineation method (Puillandre et al., 2012) to our sequence dataset. We performed ABGD analyses from the source code with default settings (JC69, Pmin: 0.001, Pmax: 0.1, steps: 10, Nb bins: 20) on each genus, and

attributed a number for each candidate species retrieved in the analysis. Computations were performed on EDB-Calc Cluster which uses a software developed by the Rocks(r) Cluster Group (San Diego Supercomputer Center, University of California, San Diego and its contributors), hosted by the laboratory "Evolution et Diversité Biologique" (EDB). In 24 instances (17 concerning Amazonian taxa), different nominal taxa in TAXO1 were lumped as a unique candidate species in TAXO2 because of shallow mtDNA divergence between them (notably in *Atelopus* spp. and *Osteocephalus* ssp.). These were considered as false negative, and the assignation that we applied in TAXO1 was then duplicated in TAXO2.

Third, we used the amphibian species range data from IUCN[1]. In order to make this dataset comparable with TAXO1 and TAXO2, we excluded 22 genera (433 species) that are partly included in our focal area but out of the scope of our study being restricted to western Amazonia, Northern Andes, Caatinga and Cerrados. One genus from the Tepuis (*Metaphryniscus*) was also omitted given no sequences were available, as well as two introduced species (*Eleutherodactylus johnstonei* and *Lithobates catesbeianus*). Overall, 51 genera were used in our analyses.

2.2.4 Study area and species distribution data

Our analyses focused on an area that included the whole central, eastern and northern Amazonia (excluding most of the western and southern parts). The limits of our study area were W 72° W 47° in longitude, and S 11° N 9° in latitude (WGS84 standard). We applied a grid of 1° by 1° (500 cells) that covered the whole area. This includes the Guiana Shield (as defined by (Lujan and Armbruster, 2011)), the central and eastern parts of the Rio Amazonas drainage, and the northern parts of the Rio Purus, Rio Madeira, Rio Tapajos, Rio Xingu, and Rio Tocantins drainages (Fig. 2.1A, B).

We then estimated the putative range of each species by creating convex polygons out of our occurrences datasets TAXO1 (358 species total within the focal area) and TAXO2 (596 species) with the *sp* package implemented in R. We then generated the occurrences of species in each cell of our study area for the three datasets, excluding species that

[1] `http://www.iucnredlist.org/technical-documents/spatial-data`

occurred in less than three localities and cells with less that five species in them. 118 species were discarded in TAXO1 and 318 in TAXO2. Finally, we considered 240 species in TAXO1, 278 in TAXO2 dataset, and 440 in the IUCN dataset (Fig. 2.1C, D, E).

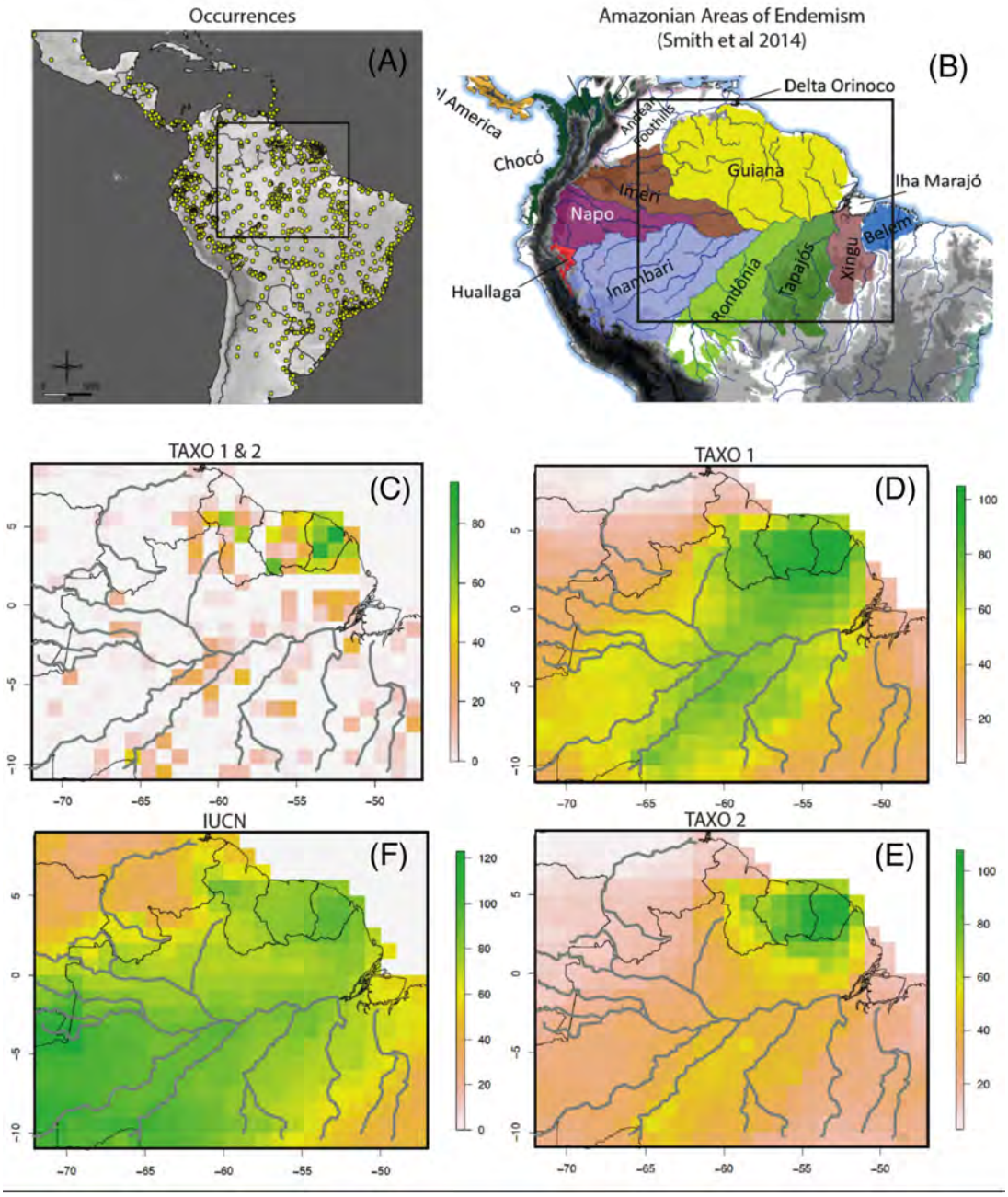

Figure 2.1 – (A) all the occurrences of the barcoding dataset and inset of the focal area; (B) Amazonian areas of Endemism from Smith et al 2014; (C) species richness mapped from occurrences data from our taxonomic framework, TAXO1 and 2 provide identical results; (D) and (E) species richness mapped from TAXO1 and TAXO2 respectively after polygon transformation (F) species richness mapped from the distribution data of IUCN considered in our analyses.

2.2.5 Identification of BSR

We decomposed the species occurrence data into several assemblages using Latent Dirichlet Allocation (LDA; see (Blei et al., 2003; Valle et al., 2014), Sommeria-Klein et al. *in prep.*). One advantage of this method over classic clustering is that it allows for modelling gradual changes in taxonomic composition over space. It is also more parsimonious than clustering algorithms based on dissimilarity metrics. The method consists in fitting a probabilistic model to the community matrix (i.e., the matrix listing the species present in each grid cell). The probabilistic model assumes that several assemblages of species coexist over the study area, the number K of which is fixed beforehand and can be optimized by AIC minimization. The assemblages may partially overlap in taxonomic composition, and a given grid cell may either be dominated by one assemblage or contain a mixture of assemblages. The estimated value of the mixing parameter α indicates whether the samples tend to be decomposed into an even mixture of assemblages (case $\alpha > 1$) or into an uneven mixture dominated by one assemblage (case $\alpha < 1$).

We used the Variational Expectation Maximization (EM) algorithm implemented by (Blei et al., 2003) and wrapped into the R package *topicmodels* (Grün and Hornik, 2011) for parameter inference, with a convergence threshold of 10^{-6} for the EM step and 10^{-8} for the variational step. We assessed the reliability of the solution by comparing the taxonomic composition of 100 realizations of the algorithm starting from random initial conditions. We only interpreted the decomposition corresponding to the realization with the highest likelihood value. We followed the approach described by Sommeria-Klein et al. (*in prep.*) for optimizing the number of assemblages, assessing the reliability of the decomposition, and representing the spatial distribution and taxonomic composition of the assemblages. We also decomposed the datasets into K=3 assemblages so as to assess the coarser biogeographic structure of the study area and test the correspondence with the Jaccard dissimilarity index.

2.3 Results

2.3.1 Underestimation of species richness

Based on our analyses, among the 363 Amazonian species found in TAXO1, 53 genetic lineages could not be associated with any nominal taxa. Most of these lineages occurring in the EGS were already documented (e.g., *Adelophryne* sp., *Scinax* sp. 2, or *Pristimantis* sp. 1) (Fouquet et al., 2007b, 2012b). However, several other lineages are reported here for the first time (e.g., *Allobates* sp. "Divisor", *Amazophrynella* sp. "Acre", *Dendropsophus* sp. "Xingú"). These are mostly from southern and western Amazonia suggesting that our sampling encompasses the large majority of the species occurring in the lowlands of the Guiana Shield but not in the rest of Amazonia. Our datasets also provide evidence of range extension for many taxa. This is for example the case of *Scinax nasicus* which extends to the Sipaliwini savannah (Suriname), *Pristimantis koheleri* to the southern part of the Guiana Shield or *Synapturanus mirandariberoi* to the southern part of the Amazonas drainage. However, most of these cases of newly documented populations are highly genetically divergent from the populations lying within the known range of the species and are considered as independent species in TAXO2.

In fact, 246 TAXO1 species display splits leading to 568 species (×2.3) in TAXO2. TAXO2 provides 1548 comparisons among species that are lumped as conspecific in TAXO1. 39 % of these average pairwise distance (p-distance pairwise deletion) were >6 % a threshold believed to conservatively delimit species (Fouquet et al., 2007a; Vences et al., 2005) and 85 % were >3 % (Fig. 2.2A). In terms of taxonomy, 436 TAXO2 species (considering the 310 nominal taxa in TAXO1) cannot be assigned to any nominal taxon in TAXO2. These observations suggest that the TAXO1 framework remains overconservative in many instances.

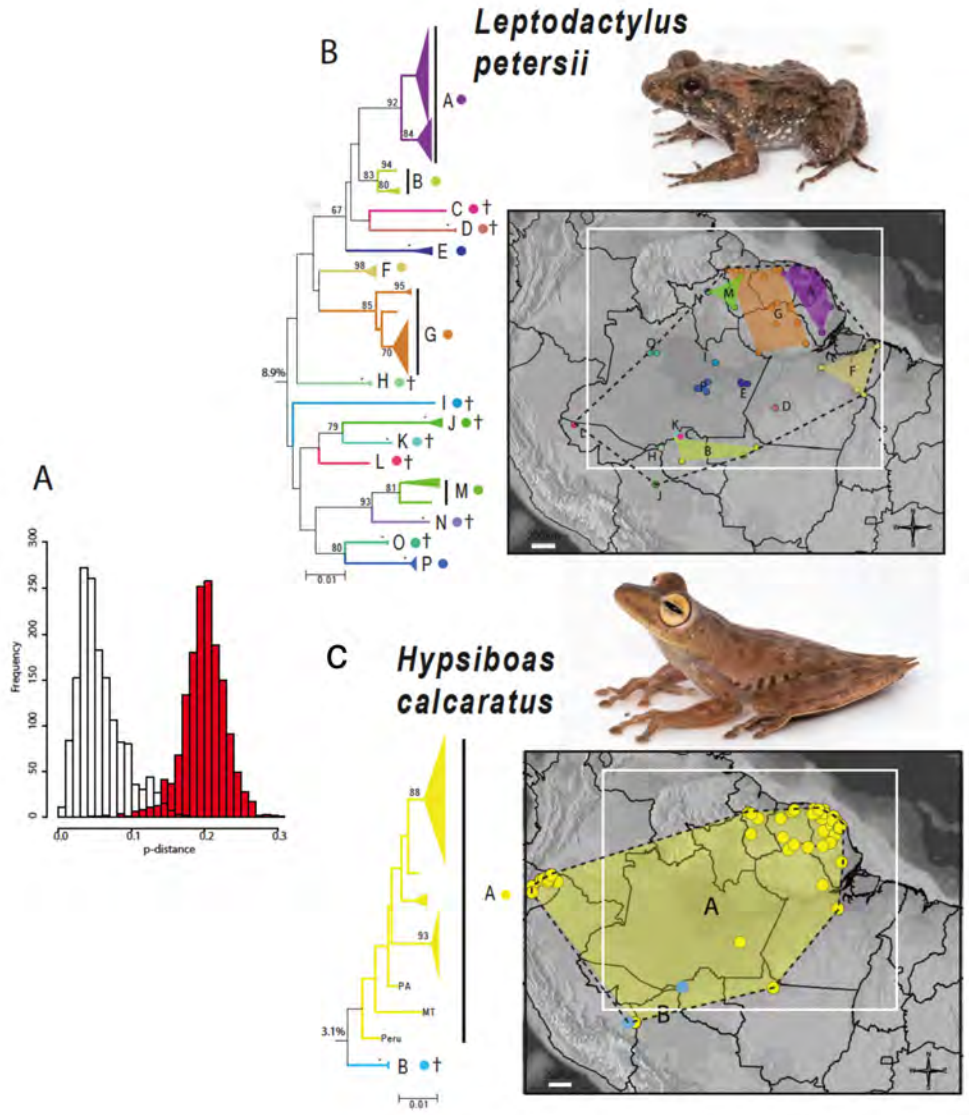

Figure 2.2 – (A) Histogram of the average pairwise distances among TAXO2 species considered as a single TAXO1 species (white bars) and among TAXO2 species considered as different TAXO1 species (red bars). This last distribution was randomly sampled to harbour the same number of comparisons than in the previous one. (B-C) Examples of genetic and geographic patterns for two Panamazonian single species in TAXO1 that provide drastically different patterns in TAXO2 (B) *Leptodactylus petersii* is split in 16 candidate species in TAXO 2. (C) *Hypsiboas calcaratus* is only split in two candidate species in TAXO2. The colours of the lineages on the tree correspond to the colours of the occurrence points and areas on the map. † indicates candidate species that were discarded from the analyses in TAXO2 (less than three locality records).

2.3.2 Distribution patterns

A number of distinct patterns of distribution emerge from the genotyped occurrence data. We highlight three of them that segregate groups of species occurring in the Guiana Shield: Guiana Shield endemic groups; Panamazonian allopatric groups; widespread

species. The first pattern concerns five groups that are endemic to the Guiana Shield and occur in both the highlands and the lowlands: *Adelophryne* (4 species in TAXO1 *vs.* 4 in TAXO2), *Otophryne* (3 *vs.* 3 species), *Synapturanus* (3 *vs.* 4 species), *Anomaloglossus* (15 *vs.* 29 species), *Vitreorana ritae* clade (3 *vs.* 3 species), *Hypsiboas benitezi* clade (3 *vs.* 3 species). Among them, only *Anomaloglossus* seems to have substantially diversified in the lowlands. Secondly, the vast majority of species occurring in the Guiana Shield lowlands are nested in widespread Amazonian or lowlands Neotropical clades (Figure 2). Most of these clades display deep divergence among populations (> 6% e.g., *Leptodactylus petersii*, Fig. 2.2B) and contain several candidate species with more restricted ranges. Finally, 78 species out of 358 (22%) with TAXO1, 45 out of 596 (8%) with TAXO2 and 142 out of 440 (32%) with IUCN actually have broad distributions (>1 millions km^2) within our focal study area (e.g. *H. calcaratus*, Fig. 2.2C).

2.3.3 Bioregions

We decomposed the TAXO1, TAXO2 and IUCN datasets using LDA. AIC minimization yielded an optimal number of species assemblages close to K=8 for all three datasets (Fig. 2.3). For comparison purpose, we chose to use K=8 for the three datasets. The retrieved assemblages were found to be spatially segregated (mixing parameter α much smaller than 1: $\alpha_{IUCN} = 0.021$, $\alpha_{TAXO1} = 0.019$, $\alpha_{TAXO2} = 0.016$) and could thus be interpreted as BSRs. The LDA decomposition was found to be reliable for the three datasets based on its stability over 100 realisations (Fig. 2.3).

Even though not identical, the spatial boundaries of the eight BSRs retrieved for TAXO1 and TAXO2 were very similar (Fig. 2.4A-B). The lowlands of the EGS were clearly separated from the rest of the study area by the Amazonas River and the Pantepui region. Moreover, the EGS was also found to exhibit some internal structure, since this area was composed of three independent BSRs, both with TAXO1 and TAXO2 and despite large differences in the distribution of the species considered (e.g., *Leptodactylus petersii*, *Lithodytes lineatus*, *Dendropsophus minutus*). One of theses three BSR (#1 on Fig. 2.4A-B) comprised the southern part of Guyana, Roraima and the Northern parts of Para and Amazonas states (Brazil). A second one (# 2 on Fig. 2.4A-B and Fig. 2.4D-E) comprised the northern part of Guyana and adjacent Venezuela. Finally, a third one (#3

on Fig. 2.4A-B and Fig. 2.4D-E) comprised the state of Amapa (Brazil), French Guiana, and Suriname. Nevertheless, the boundaries of BSR1 matched well the Rio Negro and Rio Amazonas boundaries, while it extends somewhat further west, across the Rupununi savannah in TAXO1. The boundaries between BSRs in this specific area were also much sharper in TAXO2 than in TAXO1. In the rest of the study area, there is a striking match between BSRs boundaries and Rio Madeira in TAXO1 that is already recovered for K = 3. However, both TAXO1 and TAXO2 yielded BSRs encompassing the Purus and Tapajos Rivers. The distribution of BSRs using the IUCN database provided a completely different pattern, notably not matching the EGS boundaries. The three Guianas (Guyana, Suriname, and French Guiana) were grouped together in one BSR, excluding the northwestern part of Guyana and including adjacent areas of Amapa and Para (Brazil). The southern part of the EGS was grouped with the southern part of the Amazon drainage, thus encompassing the Amazon River (Fig. 2.3C).

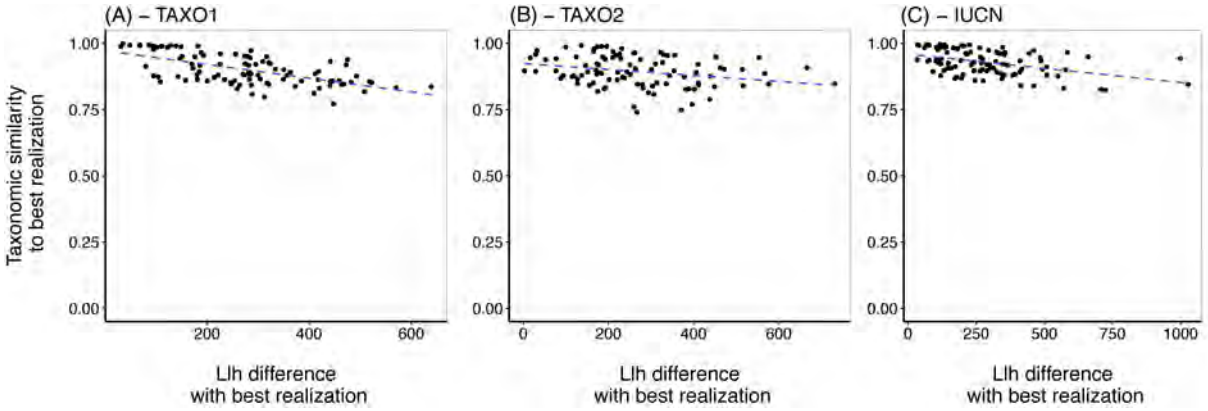

Figure 2.3 – Reliability of the LDA decomposition for 8 assemblages. Comparing 100 realisations of the LDA algorithm with random initial conditions shows, for each of the three datasets, that all realisations are similar to each other, and that they grow increasingly similar to the realisation with highest likelihood as their likelihood increases. Hence the realisation with highest likelihood can indeed be regarded as the best possible decomposition of the data.

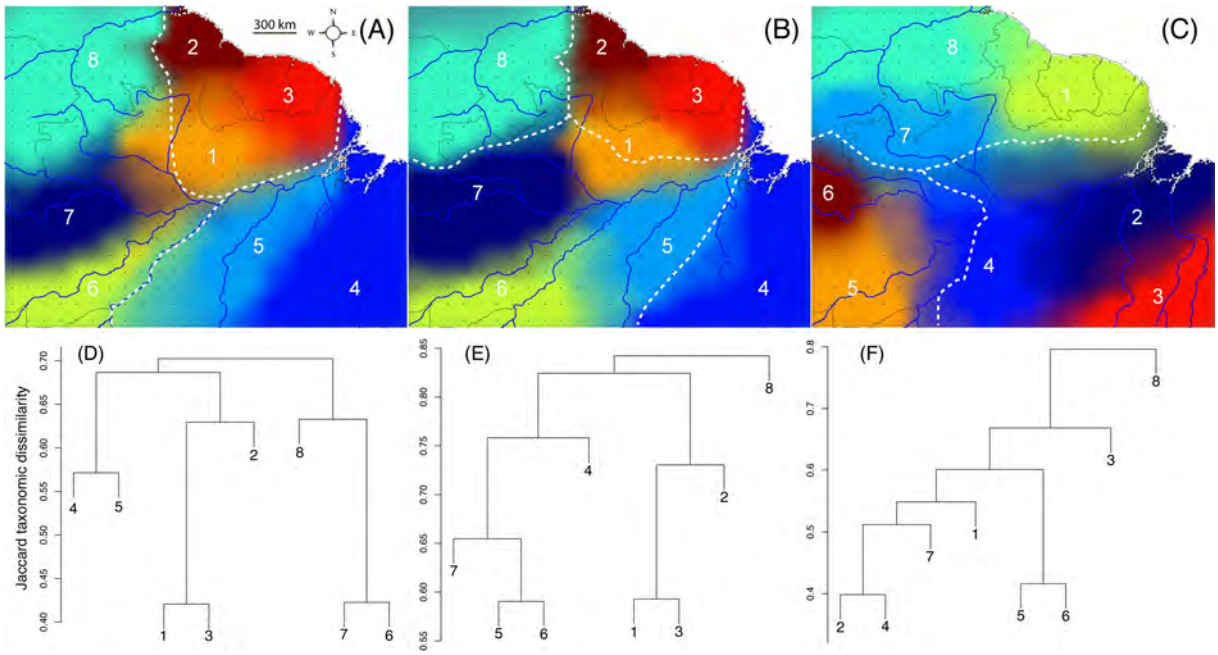

Figure 2.4 – Maps generated by interpolating the eight-assemblage Latent Dirichlet Allocation (LDA) decomposition of the species occurrence data: (A) TAXO1; (B) TAXO2; (C) IUCN data. The white dashed lines represent the approximate boundaries of the BSR for a three-assemblage LDA decomposition (in panel [C], the north-western and south-eastern regions belong to the same assemblage). Dendrograms showing the relationships between the eight assemblages recovered in the LDA decomposition using average Jaccard taxonomic dissimilarity (based on the presence/absence of species in assemblages). The numbers correspond to the numbers attributed to each assemblage for each dataset (D) TAXO1 species delineation; (E) TAXO2 ABGD species delineation; (F) IUCN data.

2.3.4 Species richness and endemism

In terms of species richness and endemism, these three datasets are radically different. The BSR1 of IUCN is composed of 119 species, 27.7 % of which are endemic (Table 2.1), and is geographically comparable to BSR2 and 3 altogether in TAXO1 and TAXO2. However, despite encompassing a smaller geographical area, BSR3 of TAXO1 alone displays similar values of richness and endemism than BSR1 of IUCN. On the scale of the three Guiana Shield BSRs, richness (184 species) and endemism (57 %) for TAXO1 are much higher than the BSR1 of IUCN. These metrics increase to 250 species and 82.4 % endemics for TAXO2 within the EGS (Table 2.1). BSR2 contains the highest number of endemic species for both taxonomic frameworks, reaching 75 % endemism for TAXO2 (Table 2.1), while the highest species richness (130) is found in BSR3 (eastern lowlands of Suriname, French Guiana and Amapa).

		UICN			TAXO1			TAXO2		
Partition	BSR	SR	ES	END	SR	ES	END	SR	ES	END
K = 3	1	–	–	–	184	105	57	250	206	82.4
	1	119	33	27.7	89	4	0.4	71	25	35.2
K = 8	2	–	–	–	85	46	54.1	90	68	75.5
	3	–	–	–	118	30	25.4	130	77	59.2

Table 2.1 – Species richness and endemism in each BSR covering the EGS. The figures presented in this table include singletons (species with only one occurrence point) and species that occur in less than three cells. BSRs numbers correspond to those displayed in Fig. 3. For K=3, assemble 1 actually corresponds to the EGS. SR = species richness; SE = number of endemic species; END = endemism (%).

2.4 Discussion

2.4.1 Underestimation of species richness and regional endemism in Amazonia

The species delineation analysis that we operated corroborates the previously highlighted perception that the actual number of anuran species occurring in Amazonia remains vastly underestimated (e.g., (Fouquet et al., 2007a; Funk et al., 2012; Ferrão et al., 2016)). The number of Amazonian species retrieved with the ABGD analysis (746) and the level of divergence among them are particularly striking in some groups (e.g., 28 CS within the *Leptodactylus podicipinus* species group in TAXO2). Our TAXO1 dataset comprised 363 species, which is close to the 440 species given by the IUCN for the same study area. However, our sampling remains low outside the EGS. TAXO1 notably does not include several nominal taxa that occur in the focal area but that are included in the IUCN database, while it includes many undescribed species that are not in the IUCN database. Therefore, the actual number of species is likely to be largely underestimated in TAXO1 outside the EGS. Moreover, the taxonomic list in TAXO1 remains over-conservative in many instances given the level of genetic divergence within species. In fact, these subdivisions have been already proven to be associated with morphologic or acoustic differences in several groups that have been included in our dataset (Fouquet et al., 2015b, 2016). For these reasons, the taxonomic framework used in TAXO2 takes into account finer subdivisions that certainly correspond to real species in many cases (see below). This dataset

suggests the existence of more than twice the number of candidate species compared to TAXO1, and thus much more than twice the current count for Amazonia. The problem of the uneven sampling is even more significant in TAXO2 as many newly delimited species remain sampled at a single or very low number of localities, suggesting that many lineages have remained unsampled, particularly outside the Guiana Shield. Hence, comparisons should be limited to the EGS where our sampling effort is the most effective. When considering solely the EGS, the number of candidate species retrieved in TAXO2 is 1.34 times higher than for TAXO1 (Table 2).

However, such a species delineation solely based on mtDNA divergence on such a broad dataset remains overly simplistic and cannot reliably delineate the species occurring in the region since it necessarily overestimates the actual number of species in some cases (false positives) and underestimates in others (false negatives) (Hickerson et al., 2006). The pitfalls inherent to the sole use of short mtDNA sequences for species delineation have been already extensively discussed (Hubert and Hanner, 2015). Nevertheless, in most groups for which the boundaries among species have been investigated using integrative taxonomy, mtDNA divergence of similar magnitude than the one used herein to differentiate between intra- and interspecific genetic divergence was generally associated with phenotypic or acoustic differentiation as well (Funk et al., 2012; Fouquet et al., 2015b, 2016; Ortega-Andrade et al., 2015). Therefore, it is highly probable that the prevalence of false positives remains low in our species delineation. In contrast, some false negatives were detected since several nominal taxa were retrieved as a single candidate species using ABGD (e.g., *Atelopus flavescens* and *A. hoogmoedi*, *O. oophagus* and *O. taurinus*). These were corrected in TAXO2 but the prevalence of false negatives remains difficult to evaluate in most groups where species boundaries have not been investigated using phenotypical traits. Overall, the present work provides an important update to the documentation of Amazonian anuran diversity, which will undoubtedly contribute to stimulate the process of species delineation and description.

If the present work provides a glimpse at how far we still are from reaching a realistic estimate of the number of species occurring throughout Amazonia, it also provides an even more striking view at the degree of regional endemism. Our estimate of the rate of endemism in frogs of the EGS reaches 57 % based on TAXO1 and 82.4 % based on TAXO2

datasets. This figure is two to four times higher than the estimates from a comparable area according to IUCN. It is also one to 1.4 times higher than the endemism of frogs estimated in the whole Guiana Shield as geologically defined, which also encompasses Venezuela and part of Colombia (Señaris and MacCulloch, 2005). In comparison, the estimates for birds are 7.7 % of endemic species in the whole Guiana Shield, 29 % for reptiles, 11 % for mammals (Hollowell and Reynolds, 2005). These figures are still certainly underestimated (Lim, 2012), especially for reptiles (Geurgas and Rodrigues, 2010; Pellegrino et al., 2011; Souza et al., 2013; de Oliveira et al., 2016; Moraes et al., 2016), but taxonomy has probably reached a much more stable level for birds and mammals in the Guiana Shield than for anurans.

A simple and rough extrapolation based on the species richness and endemism we obtained for the EGS (184–250 species with 57–82 % endemism) applied to the eight Amazonian BSR retrieved in our analysis leads to ca. 1472–2000 species in our focal area, which represent about three to five times the 440 species that are supposed to occur according to the IUCN. Enhancing data coverage in order to refine these estimations would require extensive field work in remote areas (compare to underestimation levels from other works that are similar, about 1/4 (Funk et al., 2012) or (Vieites et al., 2009)). Nevertheless, new predictive approaches based on detection of cryptic diversity (Espíndola et al., 2016) would permit to get a more precise estimate of species richness and endemism in each BSR, and therefore would help targeting areas where to focus sampling.

2.4.2 Biogeographic divisions of the Eastern Guiana Shield

The extent of the BSRs retrieved for TAXO1 and TAXO2 are very similar in spite of the use of two drastically different taxonomic frameworks. In contrast, the BSRs retrieved from the IUCN database are very different and do not correspond to any hydrological features. No barrier effect of the lower Rio Amazonas is even distinguishable. This is most likely resulting from the artifactualy large distribution of many species contained in this database on both sides of this river.

The location of the Rio Madeira matches well the boundary between BSR5 and 6 in TAXO1, which is in accordance with what has already been shown for other groups of

terrestrial vertebrates, such as birds (Fernandes et al., 2012; Ribas et al., 2012) and primates (Cortés-Ortiz et al., 2003). The sharpness of this pattern is not obvious in TAXO2, but this is unambiguously due to the removal of many singletons from the dataset after the species delineation. Another interesting aspect is the lack apparent suture effect of the Purus and the Solimoes drainages, also in accordance with what have been previously found for other group of terrestrial vertebrates (Cortés-Ortiz et al., 2003; Fernandes et al., 2012; Ribas et al., 2012). These rivers display a meandering behaviour associated with an unstable course over time, thus enabling gene flow through connection between populations located on both their sides and dispersal of species from one interfluve to the other (Aleixo, 2004, 2006; Bates et al., 2004; Antonelli et al., 2010). On the contrary, wide rivers in the Brazilian shield such as Rio Madeira display a putatively more stable course over time and are more likely to act as long lasting suture zones that might have promoted diversification or at least been more efficient in preventing dispersal (Antonelli et al., 2010; Moraes et al., 2016). Such characteristics are also found in rivers of the Guiana Shield (Fernandes et al., 2012; Fouquet et al., 2012a, 2015a), but except for the Rio Branco and Rio Negro, the impact of the Guiana Shield rivers on gene flow through limitation of dispersal might not be as important as Amazonian rivers of the Brazilian Shield, due to the smaller extent of the catchments and the smaller width of the rivers themselves. This is reflected in our results, as the suture zones between the three BSRs of the EGS do not correspond to any major drainage. In fact, it is more likely that the delimitation of these assemblages resulted from combined influences of past climatic and landscape changes. The current climatic characteristics of the EGS are heterogeneous, with a large dryer corridor observed in the southern part (Mayle and Power, 2008), where patches of savannahs are found today. This corridor also matches the suture zone between BSR1 vs. 2 and 3. The strong climatic fluctuations in the Neotropics during the Miocene and Pliocene played a crucial role in the diversification of several organisms (Antonelli et al., 2010). More recent climate fluctuations and associated landscape modifications during the Pleistocene certainly helped maintain the diversity that resulted from diversification events from the earlier Miocene/Pliocene period (Carnaval and Bates, 2007).

The outer limits of the three BSRs that constitute the lowlands of the Guiana Shield matches well with the delimitation of the Guianan area retrieved for birds (Naka, 2011), confirming the relevance of qualifying the eastern Guiana Shield as a biogeographic unit.

Nonetheless, using anuran assemblages as models revealed biogeographic heterogeneity within this region that could not be detected with birds assemblages, likely because birds have much higher dispersal abilities than anuran amphibians (Pigot and Tobias, 2015). The distinctiveness of the Guiana Shield BSRs compared to the remaining of the dataset is also reflected in the structure of the dendrogram illustrating the level of taxonomic similarity between assemblages (Fig. 4). The southern limit of BSR1 corresponds to Rio Amazonas for both TAXO1 and TAXO2 datasets. This is congruent with previous studies on terrestrial vertebrates indicating that Rio Amazonas is a strong barrier to gene flow and that assemblages are not similar north and south of this river (Cortés-Ortiz et al., 2003; Haffer, 2008; Ribas et al., 2012). The delineation of the western part of BSR1 differ across datasets; it coincides perfectly with the lower Rio Negro, and the Rio Branco and associated savannahs (Rupununi) in TAXO2 but extends further west in TAXO1. These differences are inherent to the scarcer the sampling west and south west of the Rio Negro and Rio Branco weakening the sharpness of the analysis in that zone which become even more prevalent in TAXO2 because of the taxonomic subdivisions. Another reason could be the inclusion of both forest and open habitats species in our analysis that could blur the pattern in areas where savannah and forest are found.

It is interesting to note that the limits of the BSRs of the EGS are rather similar when considering either a K=3 or a K=8 decompositions, for both TAXO1 and TAXO2. This indicates that a strong co-occurrence signal underlies the delineation of these BSRs, especially in the case of the two northernmost ones (2 and 3) whose western and eastern boundaries coincide perfectly with the ones retrieved in the three-assemblage decomposition (Fig. 3).

2.4.3 Conclusion

Despite being far from exhaustive our barcoding dataset is the largest ever gathered for Amazonia, and we argue that it is close from exhaustive in the EGS. Of course, the pattern we obtained needs to be confirmed in other taxonomical groups and even need to be much improved for anurans outside the eastern Guiana Shield. However, our results help understanding the spatial scale of sampling efforts needed to capture the actual diversity in Amazonia. The magnitude of the Linnean and Wallacean shortfalls in Amazonia is

so large that we could question the conclusions of large scale studies based on currently admitted biodiversity data in Amazonia (Feeley and Silman, 2011; Foden et al., 2013). In fact, even with very coarse data (IUCN), they estimated that Amazonian amphibians are highly threatened by climate change. Considering that most species were not included and that they actually harbour much narrower distributions, we can hypothesise that the situation is even more worrying. If a degree of endemism similar to the one we estimated within the EGS actually occurs across Amazonia, the impact of habitat loss could have been underestimated. It is especially the case along the Arc of deforestation (Vedovato et al., 2016), given entire faunal assemblages that may harbour a high degree of endemism are at risk of extinction (Da Silva et al., 2005).

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Chapter 3

Cryptic diversity in Amazonian frogs: Integrative taxonomy of the genus *Anomaloglossus* (Amphibia: Anura: Aromobatidae) reveals a unique case of diversification within the Guiana Shield

Foreword to Chapter 3

This work was the subject of an article that has been submitted to the journal *Molecular Phylogenetics and Evolution*, and accepted for publication with minor revisions. It is co-authored with Antoine Fouquet, Philippe J.R. Kok, Miguel Trefaut Rodrigues, Jucivaldo Dias Lima, Andy Lorenzini, Quentin Martinez, Manon Fallet, Elodie A. Courtois, Michel Blanc, Philippe Gaucher, Maël Dewynter, Rawien Jairam, Paul Ouboter, and Christophe Thébaud.

3.1 Introduction

Most of the diversity of terrestrial extant organisms is found in the tropical mountains and tropical forests (Antonelli and Sanmartín, 2011; Dahl et al., 2009; Jenkins et al., 2013; McInnes et al., 2013). However, in vast regions largely covered with forests like Amazonia, estimates of basic metrics of biodiversity such as the number of species occurring at the regional scale as well as data on the distribution of species still remain very vague in many taxonomic groups (Bickford et al., 2007; Fouquet et al., 2007a; Vieites et al., 2009). Overcoming the lack of knowledge on species identities and distribution is particularly challenging in groups with apparently high levels of cryptic species diversity (*i.e.*, two or more species classified as a single nominal species) because they are at least superficially morphologically indistinguishable (Bickford et al., 2007). Among vertebrates, amphibians are a group in which the occurrence of morphologically cryptic species appears to be rather common, as suggested by recent studies across the three extant orders (Fouquet et al., 2007b; Díaz-Rodríguez et al., 2015; Fouquet et al., 2014; Funk et al., 2012; Gehara et al., 2014; Kok et al., 2016a,b; Nishikawa et al., 2012; Stuart et al., 2006; Vieites et al., 2009; Wielstra et al., 2013; Wielstra and Arntzen, 2016). Amphibians tend to show evolutionary conserved morphologies, certainly promoted by the use of non-visual reproductive signals (calls, pheromones) (Bickford et al., 2007; Cherry et al., 1982; Emerson, 1988), and therefore some groups harbor few discernable morphological taxonomic descriptors. Integrative approaches, especially those combining morphological, bioacoustic and molecular data have proved to be particularly useful to clarify the taxonomic status of lineages containing high level of cryptic species diversity and to reveal previously undetected diversity (Padial and de la Riva, 2009; Simões et al., 2013; Vieites et al., 2009).

The genus *Anomaloglossus* is one of these challenging amphibian groups in terms of species delineation, due to large intraspecific morphological variability and the lack of morphological characters allowing easy diagnosis among species (Grant et al., 2006; Kok, 2010). Recent molecular analyses have revealed several deeply divergent lineages within currently recognized species (Fouquet et al., 2007b, 2012b; Kok et al., 2012), raising the possibility that these species may harbor multiple morphologically cryptic species. *Anomaloglossus* currently comprises 26 described species, and forms a well-defined clade of terrestrial frogs endemic to the Guiana Shield (GS) (Fouquet et al., 2015; Frost, 2016;

Santos et al., 2009). Five additional species reported from the Chocó region in Ecuador, Colombia and Panama are still allocated in the genus even though they are not closely related to any of the species currently recognized in the GS (Grant in Fouquet et al. 2015). While *Anomaloglossus* is endemic to the GS, most species (20) are found in the highlands of the Pantepui region, in the western GS, whereas the remaining six species are distributed outside Pantepui, mostly throughout the upland and lowland forests of the eastern part of the GS (EGS) (Barrio-Amorós et al., 2010; Kok and Kalamandeen, 2008; Lescure and Marty, 2000; Ouboter and Jairam, 2012). Compared to other amphibians, *Anomaloglossus* seems to be the only genus to have significantly diversified throughout the GS; all other groups restricted to the GS seem to have diversified more locally, either in Pantepui (*e.g.*, *Oreophrynella*, *Stefania, Myersiohyla*) or only within the Amazonian lowlands. As a corollary, *Anomaloglossus* species generally have very small ranges, often restricted to one or few mountainous massifs (e.g., Barrio-Amorós et al. 2010; Barrio-Amoros and Santos 2011; Kok et al. 2010, 2013; Señaris et al. 2014). This microendemic distribution pattern is also displayed by two species outside the Pantepui region, *A. apiau* (Serra do Apiau in Roraima state, Brazil) and *A. leopardus* (Apalagadi Mountains in southern Suriname). In contrast, the four other species of the EGS are considered to have broader ranges. *Anomaloglossus degranvillei* occurs in most of French Guiana (Lescure and Marty, 2000), *A. baeobatrachus* in Suriname, French Guiana, and the states of Pará and Amapá in Brazil (Avila-Pires et al., 2010; Fouquet et al., 2012b; Lescure and Marty, 2000; Ouboter and Jairam, 2012), *A. surinamensis* in Suriname and French Guiana (Fouquet et al., 2012b; Ouboter and Jairam, 2012), and *A. stepheni* from the Amazonas state in Brazil to Suriname (Avila-Pires et al., 2010; Fouquet et al., 2012b; Hoogmoed, 2013). Our current understanding of the distribution of these large range EGS species might be erroneous because several deeply divergent lineages have been uncovered among populations of *A. surinamensis*, *A. degranvillei*, and *A. baeobatrachus* (Fouquet et al., 2007a, 2012b).

Moreover, *Anomaloglossus* species display striking variation in reproductive modes: endotrophic and nidicolous in *A. stepheni* (Junca et al., 1994), endotrophic and phoretic in *A. degranvillei* (Lescure, 1975), exotrophic with maternal care in *A. beebei* (phytotelm-breeder), *A.kaiei*, and *A. roraima* (phytotelm-breeder) (Kok et al., 2006a,b, 2013), and exotrophic and phoretic in other species that have been documented (Grant et al., 2006).

In order to better understand the patterns of diversity, distribution, and reproductive traits in lowland *Anomaloglossus*, we tested species boundaries combining molecular, morphometric, bioacoustics and natural history data.

3.2 Material and methods

3.2.1 Collecting data in the field

We collected specimens during various trips in French Guiana and Suriname, as well as in the Roraima, and Amapa states in Brazil. Specimens were searched actively during the day, and caught by hand. They were euthanized by injection of a solution of lidocaine immediately after being photographed, fixed in 10% formalin for 24 hours and then transferred in 70% ethanol for permanent storage. When possible, calling males were recorded before collection (see below *Bioacoustic data*). Whenever possible, we also gathered data on habitat (*terra firme* forest vs. stream banks), and on tadpole development mode by collecting tadpoles and examining the buccal morphology in order to determine if they were endotrophic or exotrophic.

3.2.2 Molecular data

We extracted DNA from liver tissue of 258 samples using the Wizard Genomic extraction protocol (Promega; Madison, WI, USA), and we amplified a fragment of the 16S rDNA of the mitochondrial DNA. PCR were conducted in a final volume of 25 μl each containing 2 μl of DNA template, 14.36 Mq water, 5 μl of 10 x PCR Buffer, 1.25 μl of each primer, 1.67 μl of MgCL2, 0.5 μl of dNTPs, and 0.22 μl of GoTaq (Promega, Madison, Wisconsin, USA). The PCR conditions were as follows: 8 cycles of denaturation (45 s at 94°C), annealing (60 s at 46°C), and elongation (90 s at 72°C), followed by 22 cycles of denaturation (45s at 94°C), annealing (60 s at 50°C), and elongation (90 s at 72°C). For 16S rDNA, we used N16F and N16R primers (Salducci et al., 2005). Sanger sequencing of that fragment of 16S rDNA of 183 samples was performed by Genoscreen (Lille, France). The 75 remaining 16S rDNA sequences were obtained through MiSeq sequencing (Illumina, USA). We collated these sequences with all 16S sequences of *Anomaloglossus* available from GenBank (n = 244). The final 16S dataset contained sequences of 502 specimens of

Anomaloglossus.

Additionally, we amplified and sequenced three protein-coding nuclear loci (*tyrosinase* - TYR; *proopiomelanocortin C* -POMC; and *recombination activating gene exon 1* -RAG1). PCR conditions were similar as for the 16S fragment. For TYR, we used tyrE dendro5 and tyrE dendro primers (Fouquet et al., 2012b), for POMC we used POMC-1 and POMC-2 primers (Wiens et al., 2005), and for RAG1 we used MARTFL1 (Hoegg et al., 2004) and RAG1-AD2R (Fouquet et al., 2014) for the first fragment and RAG1-810F and RAG1-1240R (Fouquet et al., 2014) for the second fragment. We completed the dataset by adding 15 TYR sequences that were already available in GenBank.

3.2.3 Phylogenetic analyses

A first set of analyses was performed on 503 sequences of the 16S rDNA dataset alone, which were aligned with MAFFT v.7 (Katoh and Standley, 2013) using default parameters (gap opening penalty = 1.53; gap extension penalty = 0.123; progressive method = FFT-NS-2). The resulting alignment was 418 bp long after exclusion of non-overlapping regions. A XML File was generated with BEAUti v.1.8.0 with the following settings: GTR+G+I substitution model, inferred as the best fitting model with PartitionFinder v.1.1.1 (Lanfear et al., 2012) with a Bayesian information criterion (BIC), empirical base frequencies, four gamma categories, birth-death process model, all codon positions partitioned with unlinked base frequencies and substitution rates. We then performed a Bayesian analysis using BEAST v.1.8.1 (Drummond et al., 2012), with an uncorrelated relaxed lognormal clock model under default parameters. The length of MCMC chain was 50,000,000 sampling every 5000. Trace files were evaluated with Tracer v1.6.0 (Rambaut et al., 2014). Maximum clade credibility trees with a 0.5 posterior probability limit, and node heights of target tree were constructed in TreeAnnotator v1.8.1 (Rambaut and Drummond, 2012).

A second set of molecular analyses was performed using the three nuclear protein-coding loci. These data were used to examine the congruence between mtDNA and nuDNA given that reciprocal monophyly of the same sets of individuals can be seen as evidence of reproductive isolation, particularly when there is an overlap among their ranges. We used 48 samples, and missing data were limited to only one locus per terminal (three terminals for POMC, five terminals for RAG1, and five terminals for TYR). MEGA

v.7.0.16 (Kumar et al., 2016) was used to align sequences of each locus and to review amino acid translations to ensure correct alignment with respect to reading frame. We then used the program FASconCAT v.1.0 (Kück and Meusemann, 2010) to concatenate the three fragments because each locus was individually recovered as poorly informative in preliminary analyses. The resulting alignment comprised three partitions of a total length of 2524 bp (POMC 1–605, RAG1 606–2002, TYR 2003–2524). We inferred the best-fitting model of molecular evolution with PartitionFinder v.1.1.1 (Lanfear et al., 2012) with BIC, and conducted a ML analysis with RAxML v.8.2.4 (Stamatakis, 2014) using the GTR+Γ model. Support of nodes was investigated with 1000 bootstrap replicates using the fast bootstrapping algorithm. For both analyses, *Ameerega hahneli*, *Mannophryne collaris, Rheobates palmatus*, *Allobates femoralis*, *Allobates olfersioides*, and *Aromobates saltuensis* were used as outgroups (Santos et al., 2009). Mean pairwise *p-distances* were calculated among the main lineages with MEGA v.7.0.16 using pairwise deletion.

Computations were performed on EDB-Calc Cluster which uses a software developed by the Rocks(r) Cluster Group (San Diego Supercomputer Center, University of California, San Diego and its contributors), hosted by the laboratory "Evolution et Diversité Biologique" (EDB).

3.2.4 Species delineation

Because our dataset was unbalanced in terms of number of specimen per species, we applied three different methods of DNA-based species delineation on the 16S rDNA dataset: Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012), and two phylogeny-aware methods, General Mixed Yule Coalescent (GMYC) (Monaghan et al., 2009; Pons et al., 2006), and Poisson-Tree Process (PTP) (Zhang et al., 2013).

We ran the GMYC analyses with the *ape* (Paradis et al., 2004) and *splits* (Ezard et al., 2009) packages implemented in R v.3.2.4 (R Development Core Team, 2016).

PTP is similar to GMYC, but it does not require an ultrametric tree and is supposed to outperform GMYC when evolutionary distances between species are small (Zhang et al., 2013), a bias which is expected in our dataset (Fouquet et al., 2012b). As a maximum likelihood (ML) tree is required for this analysis, we subjected the 16S rDNA alignment to phylogenetic inference using ML as implemented in RAxML v.8.2.4 (Stamatakis, 2014). We inferred the best-fitting model of molecular evolution with PartitionFinder v.1.1.1

(Lanfear et al., 2012) with a BIC. Support of nodes was investigated with 1000 bootstrap replicates using the fast bootstrapping algorithm, as it produces almost identical values as the standard bootstrap method but is faster (Stamatakis et al., 2008). *Mannophryne collaris, Rheobates pseudopalmatus*, and *Aromobates saltuensis* were used as outgroups (Santos et al., 2009). We then used the best ML tree (excluding the outgroups) obtained with RAxML as an input for a PTP analysis that we ran on the online PTP server (`http://species.h-its.org/ptp/`). We ran the PTP analysis using 100,000 MCMC generations, with a thinning value of 100, and a burn-in of 0.1. We performed ABGD analyses from the source code with two different distance metrics (JC69 and simple *p-distance*) using default values for all parameters (Pmin: 0.001, Pmax: 0.1, steps: 10, Nb bins: 20).

3.2.5 Morphometric data

We measured 89 male specimens assigned to the four nominal species of the *A. stepheni* species group (*A. apiau*, *A. baeobatrachus*, *A. leopardus*, and *A. stepheni*) and 56 male specimens assigned to two species of the *A. degranvillei* species group (*A. degranvillei* and *A. surinamensis*). Two populations (Acari and Paru) were not included because specimens were not available. We measured 17 variables: snout-vent length (SVL); head length from corner of mouth to tip of snout (HL); head width at level of angle of jaws (HW); snout length from anterior edge of eye to tip of snout (SL); eye to naris distance from anterior edge of eye to center of naris (EN); internarial distance (IN); horizontal eye diameter (ED); interorbital distance (IO); diameter of tympanum (TYM); forearm length from proximal edge of palmar tubercle to outer edge of flexed elbow (FAL); hand length from proximal edge of palmar tubercle to tip of finger (HAND); width of disc on Finger III (WFD); tibia length from outer edge of flexed knee to heel (TL); foot length from proximal edge of inner metatarsal tubercle to tip of toe IV (FL); width of disc on Toe IV (WTD); thigh length from vent opening to flexed knee (ThL); length of Finger I from inner edge of thenar tubercle to tip of disc (1FiL) following (Fouquet et al., 2015), except TYM in species of the *A. degranvillei* species group because the tympanum is inconspicuous in these taxa. All measurements were taken on preserved specimens using a digital caliper to the 0.1 mm.

3.2.6 Bioacoustic data

We recorded specimens during various field trips in the EGS. Material used for call recording includes Olympus LS11 and Zoom H4N digital recorders, attached to a Sennheiser ME-66 supercardioid microphone powered with a K6P module. We analyzed call recordings of 55 males assigned to three nominal species of the *A. stepheni* species group (*A. baeobatrachus, A. leopardus, A. stepheni*) and of 31 males assigned to two nominal species of the *A. degranvillei* group (*A. degranvillei* and *A. surinamensis*). *Anomaloglossus apiau* was excluded from this analysis because this species displays a temporal call structure (long trills of paired notes) significantly different from the other species of the *A. stepheni* group (short trills of single notes). No call recording was available for populations from Acari and Paru. For species the *A. stepheni* group, which emit a train of pulsed notes, we measured six call variables using Audacity v.2.1.1. Variables follow those standardized in (Kok and Kalamandeen, 2008): Call rate (number of calls divided by their window duration), call length, note length, inter-note interval, note repetition rate (note rate: call duration divided by the number of notes in the call), and the dominant frequency (Harm2nd freq). For the *A. degranvillei* group, which emits single note calls, we considered three variables (note length, internote length, Harm2nd freq). For each variable per individual we used the mean value calculated across four different calls.

3.2.7 Data visualization and statistical analyses

We examined independently morphometric and bioacoustic data for the two species group through principal component analysis (PCA), in order to visualize relationships among data (James and McCulloch, 1990). To control for variation in body-size among individuals, we additionally performed subsequent analyses on a size-corrected dataset obtained by linear-regressing the original morphometric measures of each variable with SVL (Strauss, 1985). For bioacoustics characters, we repeated the analyses considering solely the groups of individuals that were overlapping in the preliminary analyses and removed *A. apiau* because its call has a temporal structure distinct from calls of other species in the *A. stepheni* group.

In order to test if the variable "species" would explain the variance of the data, we performed a permutational non-parametric multivariate analysis of variance (Anderson, 2001) on each set of data (morphometrics and bioacoustics for both species groups). All

analyses were conducted with the software R v.3.2.4 (R Development Core Team, 2016) with the packages *ade4* (Dray and Dufour, 2007) and *vegan* (Oksanen et al., 2016).

3.2.8 Integrative solution

In order to reach a diagnostic species delineation [*i.e.*, classify each candidate species (CS) as a confirmed candidate species (CCS), unconfirmed candidate species (UCS), or deep conspecific lineage (DCL)], we followed the framework presented by (Padial et al., 2010). We considered as "confirmed" any CS for which there was at least one congruent difference in any other character than the primary molecular divergence criterion between close relatives, and as "unconfirmed" any CS for which additional data was lacking. When we observed only molecular divergence even though additional data were available, we considered these CS as "deep conspecific lineage".

3.3 Results

3.3.1 Phylogenetic analysis

Bayesian analysis of the 16S rDNA resulted in a poorly resolved tree for deep divergences, but unravelled previously undocumented diversity within described species (Fig. 3.1A). Low resolution at the base of the tree probably explains the position of *Anomaloglossus stepheni* apart from the lowland species.

One clade contains species from the Pantepui region, and the other clade is formed by species from the EGS (*A. apiau*, *A. leopardus*, and *A. baeobatrachus*). *Anomaloglossus baeobatrachus* is recovered paraphyletic with respect to *A. leopardus*. Also, five geographically distinct and well-differentiated lineages currently assigned to *A. baeobatrachus* are recovered. These lineages are represented by the populations from Serra do Acari in Para, Brazil (*p-distance* $> 6.8\%$), Paru (*p-distance* $> 3.9\%$), Mitaraka in French Guiana (*p-distance* $> 3.4\%$), Bakhuis Mountains in Suriname (*p-distance* $> 6\%$), and Brownsberg in Suriname (*p-distance* $> 3.9\%$) (Fig. 3.1B).

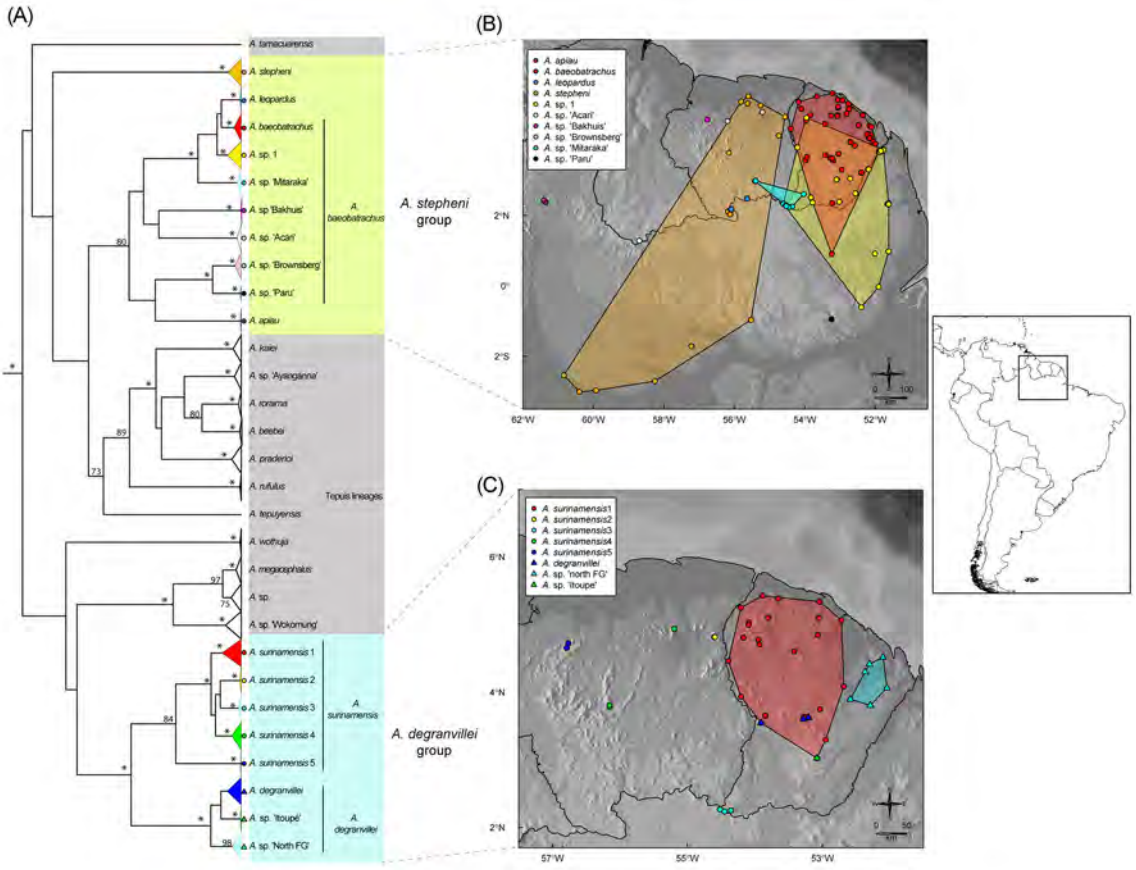

Figure 3.1 – (A) Maximum credibility clade tree obtained with BEAST. Posterior probabilities are indicated above nodes (*=0.99 or 1; not indicated when <0.7). Branches within main lineages were collapsed to indicate major clades and corresponding colour codes. (B) Map of the Eastern Guiana Shield showing the distribution records of the main lineages recovered from the phylogenetic analysis within the *A. stepheni* group and ranges of *A. stepheni*, *A. baeobatrachus*, *A.* sp.1, and *A.* sp. "Mitaraka". (C) Map of the Eastern Guiana Shield showing the distribution records of the main lineages recovered from the phylogenetic analysis within the *A. degranvillei* group and ranges of the *A. surinamensis* 1 and *A.* sp. "north FG" lineages. Inset: location of the Eastern Guiana Shield in South America.

The *A. degranvillei* group is strongly supported (pp > 0.99), as well as two subclades within it represented by *A. degranvillei* and *A. surinamensis* populations (respectively pp = 0.84 and pp > 0.99). The divergences within *A. surinamensis* are deep, in particular for *A. surinamensis* 5 in Fig. 3.1A from Bakhuis Mountains (Suriname), which forms a well-differentiated lineage (*p-distance* >6.6%) recovered as the sister group of all other representatives of this clade with good support. The remaining populations assigned to *A. surinamensis* form at least four well-differentiated lineages (*p-distances* 2.7–6.4%). These lineages are distributed allopatrically throughout Suriname and French Guiana (Fig. 3.1C). The divergences within *A. degranvillei* are lower but three lineages are discriminated (*p-distances* 1.9–2.6%). These lineages are only found in French Guiana

and on very localised massifs, except one lineage slightly more broadly distributed in north-eastern French Guiana (*A.* sp. "north FG" in Fig. 3.1C).

Even though less complete than the mtDNA dataset, the nuDNA data provides informative results about deeper relationships and species boundaries in the two species groups. Species from the Pantepui region and species from the EGS form two weakly supported clades, but the monophyly of both *A. degranvillei* and *A. stepheni* groups is strongly supported (respectively 100% and 99% bootstrap support) (Fig. 2).

Within the *A. stepheni* group, most of the candidate species are also recovered as forming independent lineages. *Anomaloglossus stepheni* and *A. apiau* form a clade well differentiated from the rest of the species group, which forms a strongly supported clade. Within this clade, *A.* sp. "Bakhuis", *A.* sp. "Brownsberg", *A. leopardus*, and *A.* sp. "Mitaraka" represent clearly segregated lineages. Populations assigned to *A. baeobatrachus* form the remaining clade.

Within the *A. degranvillei* group, the two subclades formed by populations assigned to *A. surinamensis* and *A. degranvillei* are also strongly supported (both with 100% bootstrap support). *Anomaloglossus degranvillei* and *A.* sp. "north FG" are also distinguished on nuDNA. Within *A. surinamensis*, the most divergent population, *A. surinamensis* 5 from the Bakhuis Mountains, is well differentiated from its relatives. However, *Anomaloglossus surinamensis* 1 is recovered paraphyletic with respect to *A. surinamensis* 2.

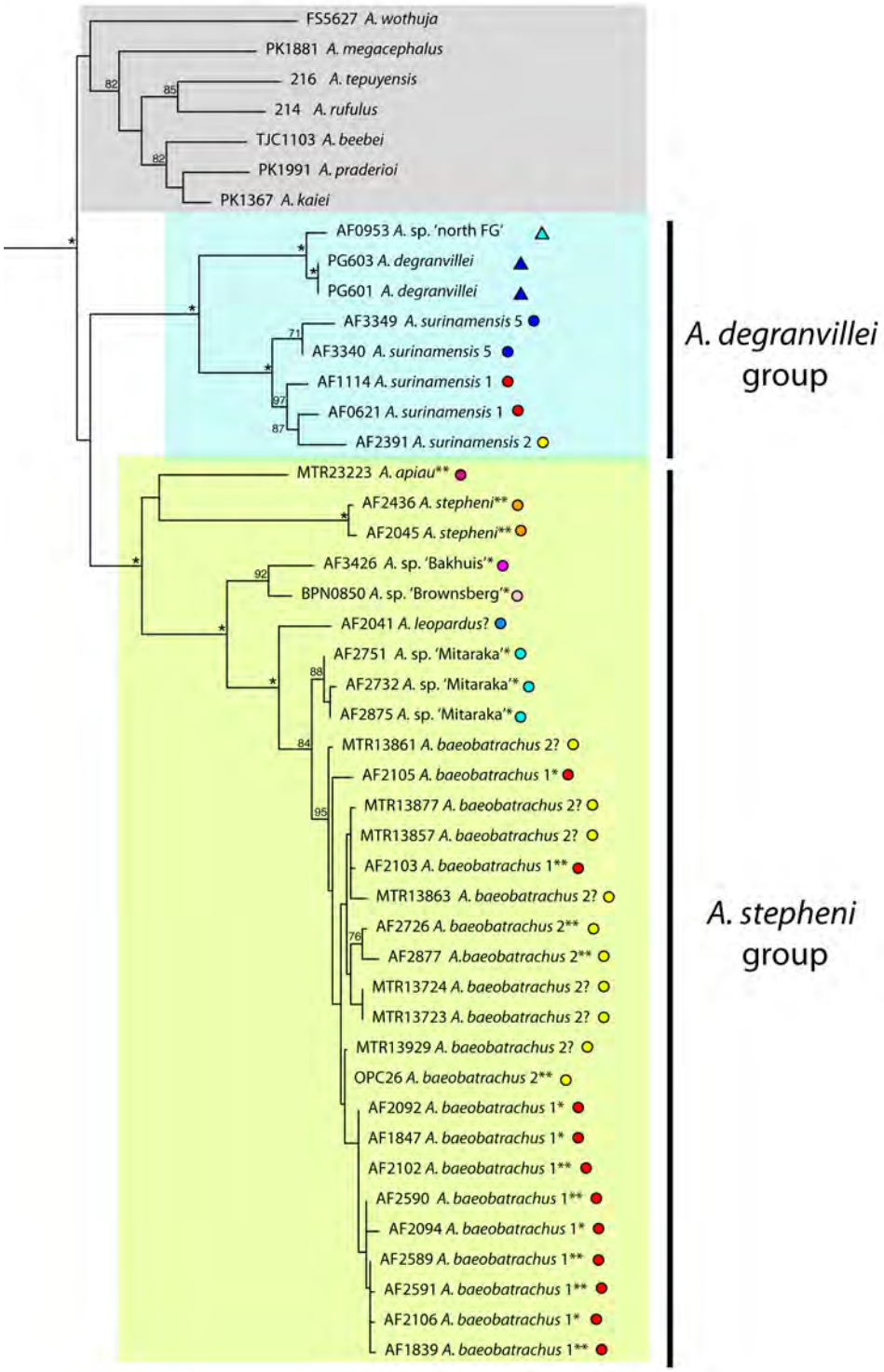

Figure 3.2 – Maximum likelihood tree obtained from concatenated nuDNA loci POMC, RAG1, and TYR. Colour codes denoted after labels correspond to the codes used in Fig. 1. Bootstrap values are indicated above nodes (* ≥99%; not indicated when <70%). For the *A. stepheni* group, we indicated *exotrophic tadpole; **endotrophic tadpole; ? tadpole development mode unknown. The coloured circle and triangle symbols correspond to the ones represented on the maps in Fig. 3.1B and 3.1C. Outgroups are not shown.

3.3.2 Species delineation

Using ABGD, a constant number of CS (31) is observed using initial partitions with a range of prior intraspecific divergence value (P=0.0046–0.001 for *p-distance*, P=0.0046–0.0028 for JC69) (Table 3.1). Recursive partitions were discarded as they provided unrealistic species delineation, notably with many singletons. For the GMYC analysis, both single and multiple threshold models outperformed the null model (Table 3.2), indicating the presence of several CS in our dataset. The result of the single threshold model (30 entities) was adopted as the fit was not improved by the multiple threshold model (chi2=2.28, df=6, p-value=0.89). The tree resulting from PTP with best-fit ML recovered 29 clusters and four singletons, resulting in a total of 33 entities. These results are summarized in Fig. 3.3.

			Prior intraspecific divergence (P)							
Subst. model	X	Partition	0.0359	0.0215	0.0129	0.0077	0.0046	0.0028	0.0017	0.001
Simple	1.5	Initial	0	13	13	16	31	31	31	31
		Recursive		14	18	20				
JC69	1.5	Initial	0	13	13	27	31	31	120	120
		Recursive		19	16	29	36	36		

Table 3.1 – Number of delimited species resulting from the automatic barcode gap discovery analysis (ABGD) with different substitution models and initial or recursive partition. X= relative gap width.

Anomaloglossus apiau, *A. stepheni*, and *A. leopardus* were identified as single CS in all analyses. However, populations currently assigned to *A. baeobatrachus* are identified as seven CS (Fig. 1B). Within these CS, two of them, *A. baeobatrachus* and *A.* sp. 1, remained indistinguishable using our nuDNA dataset (Fig. 3.2).

Eight CS were identified in the *A. degranvillei* group. Three of these CS are nested within a clade formed by populations currently assigned to *A. degranvillei*, all occurring in French Guiana (Fig. 3.1C). The remaining five CS are found in a clade formed by populations currently assigned to *A. surinamensis*, and are distributed in Suriname and French Guiana (Fig. 3.1C).

Analysis	Clusters (CI)	Entities (CI)	$\text{Likelihood}_{\text{null}}$	$\text{Likelihood}_{\text{GMYC}}$	Likelihood ratio	Threshold
Single	25 (17-33)	30 (22-39)	2965.783	2990.261	48.95***	-2.37
Multiple	35 (18-36)	40 (22-43)	2965.783	2991.401	51.23***	-1.68; -0.79; -0.15

Table 3.2 – Results of the General Mixed Yule-coalescent (GMYC) analyses for the Bayesian tree under the birth-death process model. Clusters, OTUs delineated by GMYC with more than one specimen; Entities, clusters and singleton OTUs delineated by GMYC; CI, confidence interval; $\text{Likelihood}_{\text{null}}$, likelihood of the null model; $\text{Likelihood}_{\text{GMYC}}$, likelihood of the GMYC model; Threshold, the threshold between speciation and coalescence processes. Single, single-threshold model; Multiple, multiple-threshold model; $^{***}P<0.001$.

Figure 3.3 – Multiple evidence species delimitation of *Anomaloglossus*. Results of 16S analyses in ABGD, PTP, and GMYC (from left to right, first three columns) represented on the BEAST tree, summary of all examined evidences (next five), and integrative species delimitation solution (last column). All are presented in one single topology Bayesian tree obtained with MAFFT alignment, using the birth-death process model in BEAST. Black squares represent Pantepui species (not treated here). CCS = confirmed candidate species; UCS = unconfirmed candidate species; DCL = deep conspecific lineage.

3.3.3 Morphological analyses

For both species groups, the raw morphometric data have limited discriminative power because most individual candidate species overlap with at least another one in the multidimensional space (Fig. 3.4). However, in many instances they allowed to detect some differences in body size among pairs of closely related species. Analyses performed on the size-corrected dataset confirmed the overall lack of differences among groups in their body proportions, confirming that closely related species differ mainly in their body size.

PCA on data from the *A. stepheni* group showed that two components with eigenvalues >1.0 accounted for 81.97% of the total inertia. Coefficients of the first component, which explains 67.53% of the variation (Fig. 4A), are highly and positively correlated (Fig. 3.4A, Supplementary Table S6). The second component explains 14.44% of the variation (Fig. 3.4A). Except *A. apiau*, which is well differentiated along the second axis, individuals are spread along the first axis segregating large-bodied (mean SVL>17.4 mm) species (*A. leopardus*, *A.* sp. "Bakhuis", *A.* sp. "Brownsberg", and *A.* sp. "Mitaraka") from small-bodied (mean SVL<17.4 mm) species (A. *stepheni*, *A. apiau*, *A. baeobatrachus*, and *A.* sp. 1). This was supported by the multivariate analysis of variance (MANOVA) that indicated that species identity explains 80% of the variance for morphometrics variables (Adonis MANOVA R^2=0.8, p=0.001), but only 55% when the data were corrected by body size (SVL) (Adonis MANOVA R^2=0.55, p=0.001). When the data were corrected according to body size, all the groups largely overlap except *A. stepheni*, indicating that body proportions in that species differ from those observed in other species (see suppl. mat.). Interestingly, *A. baeobatrachus* is forming two different non-overlapping clusters of individuals differing in their body size. In fact, the distinction between large-bodied and small-bodied species seems to coincide with other traits, notably larval development (see below).

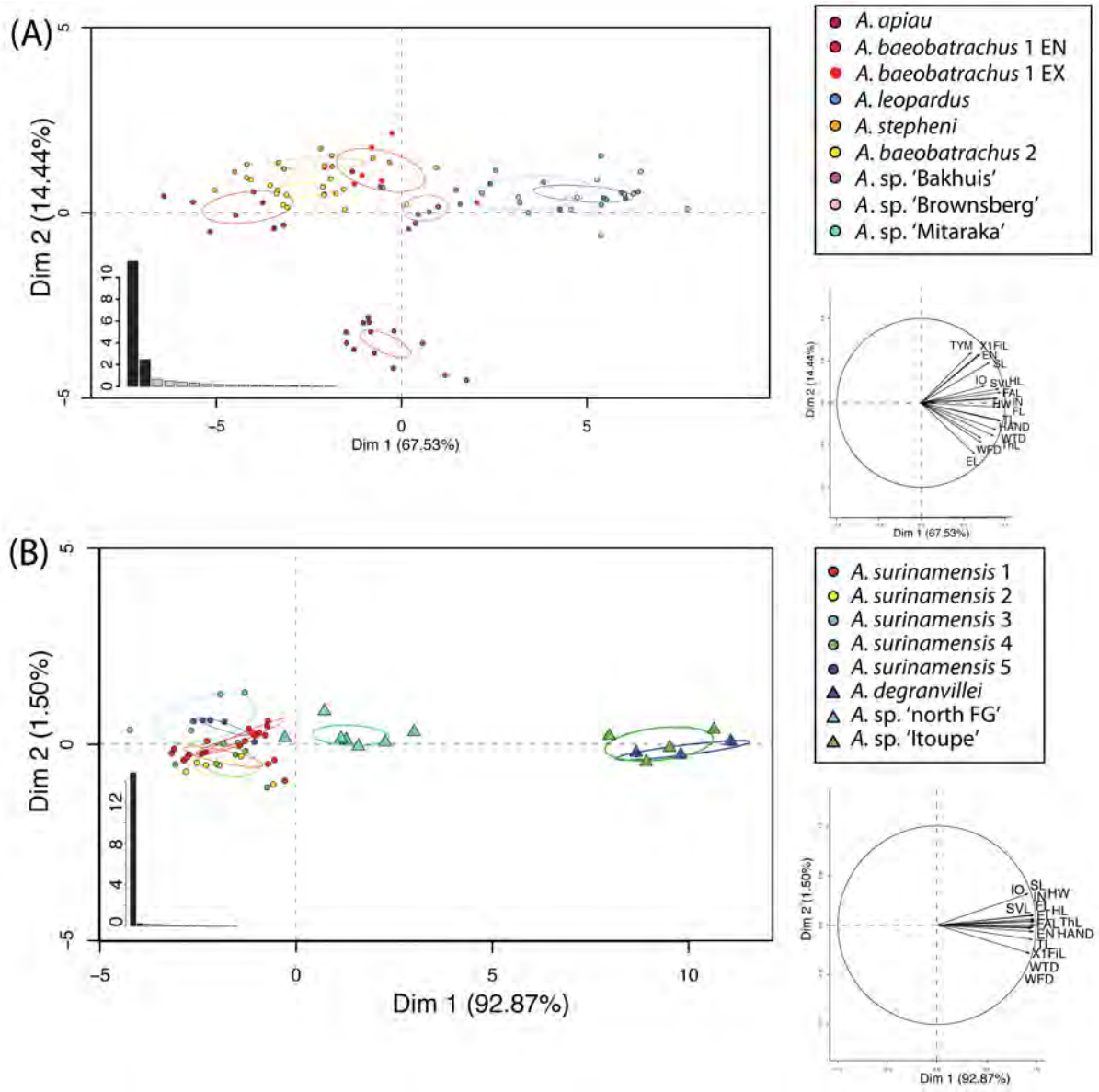

Figure 3.4 – Results of the PCA on raw morphometric variables with circle of correlations for (A) *A. stepheni* group and (B) *A. degranvillei* group. Symbols represent specimens on the first two principal components. The contribution of each axis for total variation is indicated in parenthesis. The groups are delimited with coloured lines.

For the *A. degranvillei* group, two components accounted for 94.37% of the total inertia. Coefficients of the first component, which explains 92.87% of variation (Fig. 4B), are highly and positively correlated (Supplementary Table S6). The second component explains 1.5% of variation (Fig. 3.4B), and has significant positive loading for IO and a significant negative one for WFD (Supplementary Table S6). The two subclades (*A. degranvillei* and *A. surinamensis* subclades) are well distinguished according to their body size. In the *A. degranvillei* subclade, *A.* sp. "north FG" is well segregated from the two related CS. However, the morphometric space of all CS within the *A. surinamen-*

sis subclade broadly overlaps. Additional analyses based on raw and size-corrected data focusing on the *A. surinamensis* subclade did not discriminate more groups. The multivariate analysis of variance (MANOVA) indicated that species identity explains 91% of the variance on morphometrics variables (Adonis MANOVA R^2=0.91, p=0.001), but only 29% when the data were corrected by body size (SVL) (Adonis MANOVA R^2=0.29, p=0.001).

3.3.4 Bioacoustics

Bioacoustic data showed a much greater discriminating power within the *A. stepheni* group than morphometric data with no or limited overlap among CS across the multidimensional space. Two components with eigenvalues >1.0 accounted for 82.62% of the total inertia. Coefficients of the first component, which explains 51.66% of variation, have significant positive loadings for internote length, note length, and call length, and significant negative ones for call rate, Harm2nd freq and note rate (Fig. 3.5A, Supplementary Table S7). The second component explains 30.96% of the variation, and has significant positive loadings for call rate and note length, and significant negative ones for Harm2nd freq and Call length (Fig. 3.5A, Supplementary Table S7). *Anomaloglossus baeobatrachus* contains individuals either with a slow trill call (note rate<15 notes/s) or a rapid trill call (note rate>15 notes/s) calling groups. Two distinct clusters are recovered within *A. baeobatrachus*, corresponding to the results found using morphometric data. These two groups of individuals differ markedly in their calls. Together with morphometrics, these differences seem to reflect two distinct phenotypes within *A. baeobatrachus* that occur often in sympatry (see below). These results are in accordance with the MANOVA analysis, which indicated that species identity explains 93% of on the variance on bioacoustics variables (Adonis MANOVA R2=0.93, p=0.001).

For the *A. degranvillei* group, two components accounted for 89.87% of the total inertia. Coefficients of the variables have significant positive loadings for note length and internote length, and significant negative ones for Harm2nd freq on the first component (Supplementary Table S7), which explains 62.69% of variation (Fig. 3.5B). The second component explains 27.18% of the variation, and has significant positive loading for internote length (Fig. 5B, Supplementary Table S7). The two subclades (*A. degranvillei*

and *A. surinamensis* subclades) are well segregated. In the *A. degranvillei* subclade, *A.* sp. "north FG", *A.* sp. "Itoupé", and *A. degranvillei* are all well segregated among each other. These results are in accordance with the MANOVA analysis, which indicated that species identity explains 85% of the variance on bioacoustics variables (Adonis MANOVA R^2=0.85, p=0.001). However, as for the morphometric analysis, all CS within the *A. surinamensis* subclade completely overlap. Additional analysis focusing on these individuals failed to discriminate any additional CS.

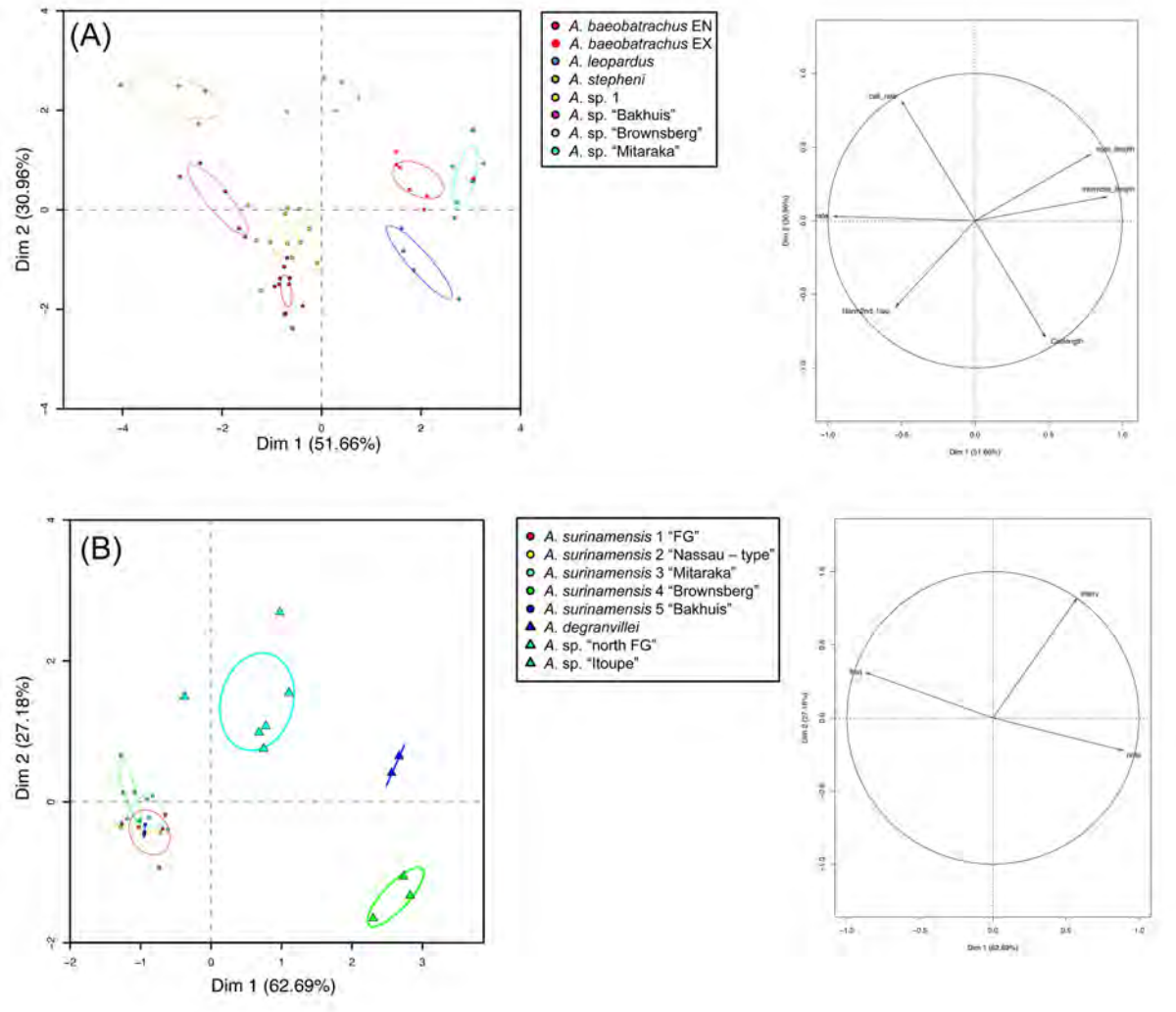

Figure 3.5 – Results of the PCA on raw bioacoustics variables with circle of correlations for (A) *A. stepheni* group and (B) *A. degranvillei* group. Symbols represent specimens on the first two principal components. The contribution of each axis for total variation is indicated in parenthesis. The groups are delimited with coloured lines.

3.3.5 Reproductive modes and habitats

In the *A. stepheni* group (excluding *A. apiau*), two reproductive modes have been observed, which seem to covary with habitat, call characteristics and body size (Table 3.3). The species in this group have either nidicolous and endotrophic tadpoles or exotrophic tadpoles with male phoresy (Fig. 3.6B). Endotrophic tadpoles are found in reduced number in the nest ($\leq$4), have reduced and bare mouth, large vitelline reserves, and complete their development in the nest. On the contrary, exotrophic tadpoles are in larger number (>4), have labial teeth, less vitelline reserves, and are transported by the male to water bodies where they complete their development.

Anomaloglossus stepheni has an endotrophic and nidicolous tadpole and is found on *terra firme* habitats (Junca et al., 1994). In contrast, *Anomaloglossus* sp. "Acari", *A.* sp. "Bakhuis", *A.* sp. "Brownsberg", *A.* sp. "Mitaraka" all have exotrophic tadpoles, that males carry to water bodies. However, both phenotypes are observed in *A. baeobatrachus* (Fig. 3.6B). Indeed, northern populations of this species are found in *terra firme* habitats and have endotrophic and nidicolous tadpoles, but some populations in the eastern and southern part of French Guiana are associated to streams, have an exotrophic tadpole and phoretic male. These populations are slightly larger in SVL and have a slower note rate. *Anomaloglossus* sp. 1 also seems to display both phenotypes. Although we could not gather totally unambiguous data, phoresy has been observed in Serra do Navio, and slow-calling individuals have been observed along the Oyapok River (Brazilian margin in Memora), Amapa state, Brazil (Grant et al., 2006), suggesting that the *A. baeobatrachus* lineage might be present in these areas.

Males carrying endotrophic tadpoles (reduced and bare mouth and large vitelline reserves) have been observed in seven populations of the *A. degranvillei* group assigned to different CS, thus documenting the mode of larval development and male behavior for most CS in that group (Table 3.3; Fig. 3.6A,C). All the members of this clade live along streams, and we assume that they display phoresy until metamorphosis, or at least during a prolonged period of the larval development. Interestingly, *A. apiau*, despite being a member of the *A. stepheni* clade, is also associated to streams and also displays this reproductive mode with tadpoles having reduced and bare mouth and large vitelline

reserves, and males transporting them until metamorphosis (Fig. 3.6C).

Data on the reproductive mode, body size, call, and habitat are completely missing for *A.* sp. "Paru", so we could not attribute a phenotype to this species. We also miss data on the reproductive mode of *A. leopardus*. However, this species is associated with streams, displays a large body size and a slow note rate, thus corresponding to phenotype 3. Therefore, it is likely that this species has exotrophic tadpoles transported by males.

Interestingly, phenotypes 2 and 3 co-occur in many places but in different combinations of CS in the *A. stepheni* group (Fig. 3.1). *Anomaloglossus stepheni* is found in sympatry with several exotrophic species in Suriname and in northern Para (Brazil) (Fig. 3.6). The two phenotypes observed in the same lineage (*A. baeobatrachus*) occur in sympatry only in the north-eastern part of French Guiana (Route nationale 2, a.k.a. RN2) (Fig. 3.6). This might also be the case in Amapa (Brazil) with two co-occurring phenotypes observed in the *A.* sp. 1 lineage. However, in most cases, the two distinct co-occurring phenotypes are observed among different lineages. The phenotype 3 of the *A. baeobatrachus* lineage occurs in sympatry with the phenotype 2 *A.* sp. 1 in the southern part of French Guiana (Fig. 3.6D) while in northern French Guiana *A. baeobatrachus* displays phenotype 2. Similarly, *A.* sp. "Mitaraka", which displays phenotype 3, occurs in sympatry with phenotype 2 of *A.* sp. 1 in south-western French Guiana. Within the *A. baeobatrachus* clade (*A. baeobatrachus* + *A.* sp. 1), phenotype 2 seems to be distributed throughout FG and Amapa (Brazil) whereas phenotype 3 is apparently absent from north-western FG (west margin of the Approuague River north of Saül). Such distribution patterns are concordant for at least seven other frog species (*Allobates granti*, *Ameerega hahneli*, *Dendrobates tinctorius*, *Engystomops* sp., *Hyspiboas dentei*, *Pristimantis gutturalis*, and *Pristimantis* sp. 3 = *A. baeobatrachus* phenotype 2 distribution pattern) and four other frog species (*Amazophrynella* sp., *Leptodactylus longirostris*, *Pristimantis* sp.1, and *Rhinella lescurei* = *A. baeobatrachus* phenotype 3 distribution pattern) (the authors pers. obs.).

Phenotype	Species	Development mode	Nidicoly Phoresy	Body size	Habitat
Phenotype 1	*A. apiau*	endotrophic	phoresy	–	riparian
	A. degranvillei	endotrophic	phoresy	–	riparian
	A. sp. "Itoupé"	endotrophic	phoresy	–	riparian
	A. sp. "north FG"	endotrophic	phoresy	–	riparian
	A. surinamensis	endotrophic	phoresy	–	riparian
Phenotype 2	*A. stepheni*	endotrophic	nidicoly	< 17.15	*Terra firme*
	A. baeobatrachus	endotrophic	nidicoly	< 17.15	*Terra firme*
	A. sp. 1	endotrophic	nidicoly	< 17.15	*Terra firme*
Phenotype 3	*A. baeobatrachus*	exotrophic	phoresy	< 17.15	riparian
	A. sp. "Acari"	exotrophic	phoresy	17.41 – 19.21	riparian
	A. sp. "Bakhuis"	exotrophic	phoresy	17.41 – 19.21	small water bodies
	A. sp. "Brownsberg"	exotrophic	phoresy	17.41 – 19.21	riparian/small water bodies
	A. sp. "Mitaraka"	exotrophic	phoresy	17.41 – 19.21	riparian

Table 3.3 – Summary of the phenotypes that are observed within the EGS *Anomaloglossus* species and the characteristics attributed to each species and phenotype.

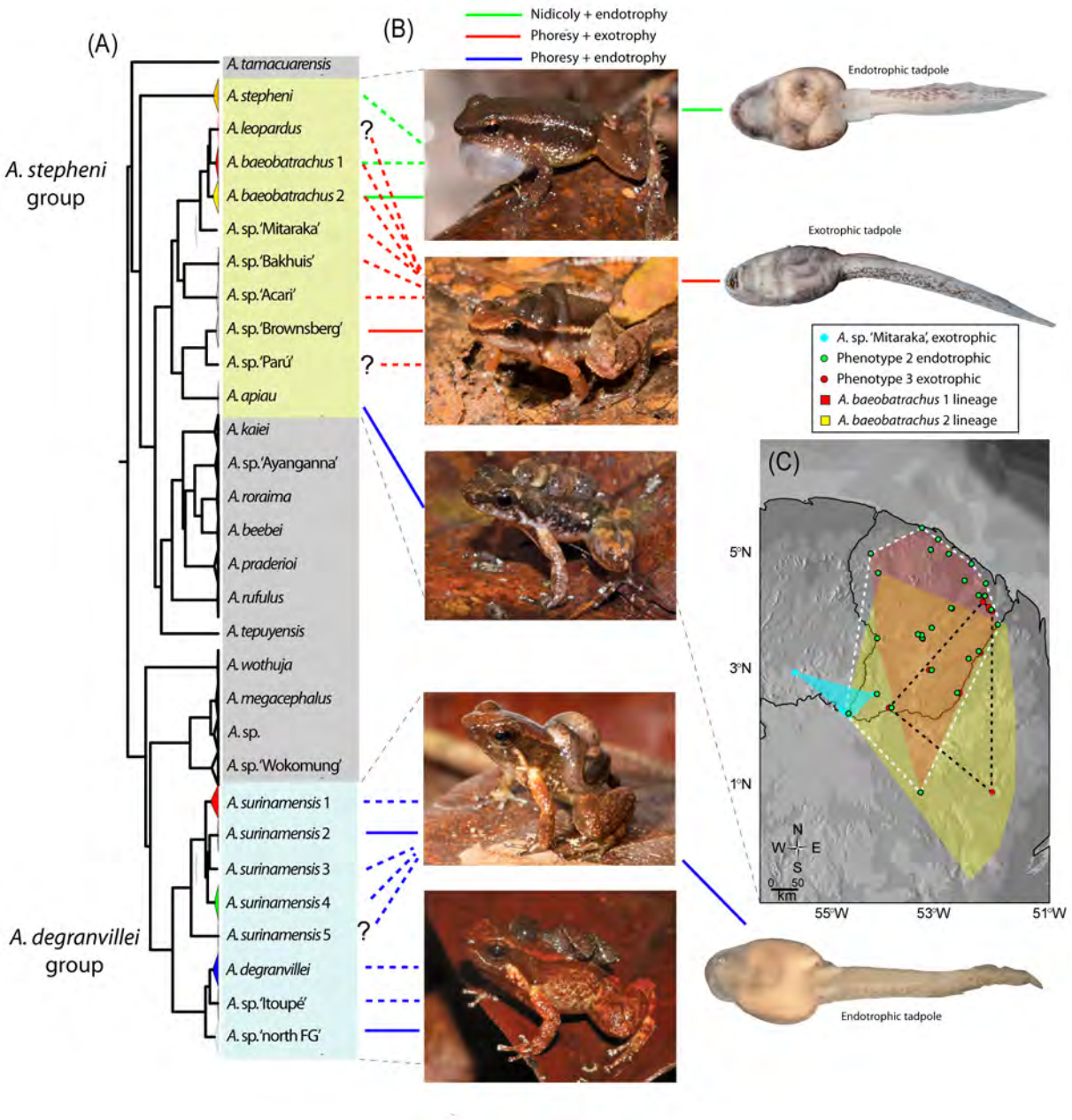

Figure 3.6 – Evidences for reproductive and larval developmental modes in the *Anomaloglossus* CS. (A) the topology obtained from analysis of the mtDNA used in Fig. 1 and 3. (B) Photographs of adult males of species representative of the different modalities (phoretic or nidicolous) found in *Anomaloglossus* [from top to bottom, the endotrophic and nidicolous *A. baeobatrachus* 2 from Mitaraka (French Guiana); the exotrophic and phoretic *A.* sp. "Brownsberg" from Brownsberg (Suriname); the endotrophic and phoretic *A. apiau* from Serra do Apiaú (Roraima, Brazil); the endotrophic and phoretic *A. surinamensis* 2 from Nassau (topotypic population in Suriname); the endotrophic and phoretic *A.* sp. "north FG" from Route Nationale 2 (French Guiana)]. Continuous lines indicate that the picture corresponds to the lineage while dashed lines indicate that the modality is found in the lineage. A question mark indicates when the modality has not been observed and is only assumed. Pictures of the three tadpoles representative of the endotrophic (reduced and non-functional mouth) or exotrophic (fully functional mouth) are also included (from top to bottom: *A. baeobatrachus* 2 from Mitaraka; *A.* sp. "Brownsberg"; *A. surinamensis* 2. The colours of the lines (blue, green, red) correspond to the three modalities of the reproductive traits found in the genus. (C) Distributions of the contrasting phenotypes in the *A. baeobatrachus* clade and the two mtDNA lineages. The white dashed line corresponds to the known distribution of phenotype 2, and the black dashed line corresponds to the distribution of phenotype 3.

3.4 Discussion

3.4.1 *Anomaloglossus* represents a unique case of *in-situ* diversification in the Guiana Shield lowlands

Our results highlight once more how far we still are from having a realistic view of the structure of the Amazonian biodiversity (Fouquet et al., 2007a; Funk et al., 2012). Underestimation of species richness within *Anomaloglossus* in the EGS had already been suggested in previous studies (Fouquet et al., 2007a, 2012b) based on more limited sampling. Our results indicate that these studies were still largely underestimating the actual diversity of the genus. Indeed, the present study identified a total of 18 lineages among populations of six currently recognized nominal species, out of which 11 are classified as CCS, six as DCL and one as UCS (three are classified as CCS and five as DCL in the *A. degranvillei* group and eight are classified as CCS, one as UCS, and one as DCL in the *A. stepheni* group). These results show that *Anomaloglossus* represents the only documented group of frogs to have significantly diversified within the Guiana Shield lowlands. Given that many of these newly discovered, yet undescribed species, are microendemics, and that many massifs in the Guiana Shield remain virtually unexplored, it is likely that more undescribed species still remain to be discovered.

Within the two main groups that are restricted to the EGS, the *A. degranvillei* group is restricted to Suriname and French Guiana, with three allopatric CCS forming the *degranvillei* subclade endemic to French Guiana, and among which two species (*A. degranvillei* and *A.* sp. "Itoupé") have a very restricted range (<500 km^2) in the southern part of the country (Fig. 3.1B). These two large-bodied species are associated to mountainous streams above 300m a.s.l., while the smaller-bodied *A.* sp. "north FG" occurs at lower elevations but is also associated to massifs. In recent years, populations belonging to these three species seem to have drastically declined and some may have gone extinct (the authors pers. obs.). It is likely that these species or additional ones occur in adjacent Suriname and Amapa state (Brazil), and given the conservation concerns raised above, they should be the focus of field surveys. The five DCL forming the *surinamensis* subclade also occur in allopatry, with three of them found in Suriname, and two others in French Guiana, but across larger areas than the *degranvillei* subclade, and no sign of decline has

been detected among these populations yet. None of the species within each subclade have overlapping ranges, but one DCL from the *surinamensis* subclade (*A. surinamensis* 1) occurs in sympatry with *A. degranvillei* and *A.* sp. "Itoupé" (Fig. 3.1B).

Within the other main group found in the EGS, the *A. stepheni* group, we were able to clarify the taxonomic status and range of *A. stepheni* and *A. baeobatrachus*. The first was recovered as diverging basally and has the widest distribution among the species group, occurring in the states of Amazonas and Para (Brazil), and in Suriname. *Anomaloglossus baeobatrachus* is restricted to French Guiana and Amapa, Brazil (Fig. 1C). Populations assigned to this species in Suriname (Ouboter and Jairam, 2012) correspond in fact to various species: *A.* sp. "Bakhuis", *A.* sp. "Brownsberg", *A.* sp. "Mitaraka", or *A. stepheni*. Out of the 10 lineages in this group, seven are localised endemics or have at least narrow ranges in the EGS (*A. apiau*, *A. leopardus*, *A.* sp. "Acari", *A.* sp. "Bakhuis", *A.* sp. "Brownsberg", *A.* sp. "Mitaraka", and *A.* sp. "Paru"), even though species occurring in poorly documented area such as northern Para (Brazil) may have larger ranges. Finally, it is highly probable that additional data (bioacoustics and reproductive mode) would allow distinguishing *A.* sp. "Paru" from its close relatives and classifying it as a CCS.

Among the rare genera that may have diversified in the GS lowlands, *Anomaloglossus* seems to be the only group of frogs to have diversified to such an extent. Many groups have diversified in the highlands of the Pantepui region (*e.g.*, *Oreophrynella*, *Stefania*, *Myersiohyla*), but none of them is closely related to lineages that diversified in the lowlands (Duellman, 1999; Kok, 2013; McDiarmid and Donnelly, 2005). Most of the lowlands lineages have apparently diversified throughout Amazonia or even larger areas throughout the continent (*e.g.*, (Fouquet et al., 2013). Among GS clades, *Otophryne* (de Sa et al., 2012), *Adelophryne* (Fouquet et al., 2012a), *Vitreorana* (Castroviejo-Fisher et al., 2014), *Hypsiboas benitezi* group (Duellman et al., 2016) all form ancient GS clades occurring in the highlands and lowlands, but all display a small diversity in the lowlands compared to *Anomaloglossus*. A pattern comparable to the one observed in *Anomaloglossus* might be found in *Pristimantis*, but available data remain too scarce.

3.4.2 Constrasting divergence patterns within *Anomaloglossus*

As expected, morphometric data provided little discriminative power in our analysis, and most CCS were mainly distinguished by acoustic data. However, within the *Anomaloglossus* species groups, we found two sharply contrasting cases of molecular and phenotypical divergence that are worth discussing, even if possible explanations remain hypothetical at this stage.

The first concerns the five CS forming the *A. surinamensis* subclade. Despite deep genetic divergence among them (ranging from 2.7% to 6.4%, see Appendix 2), none of these populations can be discriminated with any morphological or bioacoustic character. Moreover, even though sampling implied fewer individuals, congruent divergence is observed in the analyses based on nuDNA data. Since calls usually constitute strong discriminant characters among anurans (Vences and Wake, 2007), a lack of differences in calls between lineages diverging to such an extent might be surprising. Still, as they display an allopatric distribution pattern, one explanation could be that populations were isolated from each other without subsequent contacts, thus not promoting the evolution of premating isolation and therefore promoting call conservatism (Bogert, 1960; Hoskin et al., 2005). Although highly probable, this hypothesis is rather intriguing as an opposite pattern is observed within its sister group the *A. degranvillei* subclade, which occurs in similar habitat and displays similar breeding mode. The three species that compose this clade have a comparatively low genetic divergence between them (1.9–2.6 %, see Appendix 2), are also allopatric, but have well-differentiated calls. It is not clear which factor might have played a role in shaping these opposite patterns. However, we note that only the two largest species of the *A. degranvillei* subclade co-occur with *A. surinamensis* in French Guiana, while *A.* sp. "north FG" displays a similar body size as *A. surinamensis* but does not occur in sympatry with it (Fig. 3.1B). Phylogenetic relationships within the *A. surinamensis* subclade (even though deserving more investigation) demonstrated that French Guiana lineages are nested within Suriname lineages, therefore supporting the hypothesis that the *A. surinamensis* lineage could have secondarily dispersed to French Guiana from Suriname and therefore came into contact with the ancestral *A. degranvillei* subclade. One hypothesis could be that niche overlap have produced character displacement in *A. degranvillei* and *A.* sp. "Itoupé", thus evolving towards larger body size and a

more specialized niche (Brown and Wilson, 1956). As a matter of fact, these two CS are only found in torrents above elevations of 300m a.s.l., whereas at the same localities *A. surinamensis* occurs along streams below 300m a.s.l. In contrast, *A. surinamensis* and *A.* sp. "north FG" display allopatric ranges, and share a similar niche and similar body size.

The second contrasting case concerns *A. baeobatrachus* and *A.* sp. 1, two closely related lineages that both display the same two contrasted phenotypes, *i.e.*, phenotype 2 and phenotype 3. To our knowledge, such a situation has never been observed in any other group of amphibian. We think that this pattern could result from two distinct scenarios: either (1) very recent speciation in both lineages, or (2) secondary contact followed by hybridization. (1) The first scenario would suggest that given their current parapatric distribution, geographical isolation would be responsible for the divergence between these two lineages. Fouquet *et al.* (2012) estimated that such a pattern likely resulted from climate driven isolation during early Pleistocene as observed in co-occurring anuran clades. More recently, two mirroring diversification events would have occurred in each of these two species, with two converging phenotypes within each species. Subsequently, they would have dispersed and came into contact with the other pair. In this scenario, four different species (two pairs) would exist in this clade, each pair being too recent to be distinguished by molecular data (mean *p-distances* = 0.3% within *A. baeobatrachus* and 0.8% within *A.* sp. 1, see Supplementary Table S4). Such a scenario raises many questions that lie beyond the scope of the present paper. Nevertheless, it implies not only a rapid evolution of the ecology, morphology, calls, and more importantly larval development mode, but it also implies that these events would have been concomitant in the two species. (2) The second scenario would involve secondary contact between two ancestral and phenotypically distinct species. As in the previous scenario, the two lineages would have originated from historical isolation, and would have come into contact and hybridized in a recent past. Given the striking phenotypical difference currently observed despite sympatry, we would have to hypothesize that after secondary contact and formation of a hybrid zone, selection against hybrids led to the evolution of premating isolation, yet letting time for genomic exchanges, notably mitochondrial (Hoskin et al., 2005). In a second time, gene flow would have allowed exchange of introgressive genomic material in both species. This scenario raises many questions because hybridization between two species with different

larval development seems quite unlikely. Testing these two hypotheses deserve further investigation using population genomic data. It is noteworthy that either a rapid evolution of the larval development or the hybridization between species differing in larval development suggest that the genetic architecture of this trait could be rather simple and worth of further research.

3.4.3 Biogeography and evolution of reproductive modes in *Anomaloglossus*

Our results suggest that *Anomaloglossus* represents an exceptional model to study speciation and diversification in the GS. The fact that all Pantepui species apparently all have exotrophic tadpoles, while in the EGS lowlands, the *A. degranvillei* species group has endotrophic tadpoles, and the other (*A. stepheni* group) in the same area contains both modes of tadpole development suggests a strong biogeographic signal. This also reveals that endotrophy evolved several times independently in the genus. Therefore, we hypothesize that evolution towards endotrophy in this genus probably allowed populations to colonize *terra firme* environments and to disperse into new niches in the lowland forests. As a matter of fact, species displaying nidicoly associated to endotrophic tadploles (*A. baeobatrachus*, *A.* sp. 1, *A. stepheni*) have the widest distribution.

Even if endotrophy is common in anurans (McDiarmid and Altig, 1999), lineages of closely related populations or species that include both endotrophic and exotrophic developmental guilds are very rare (Anstis, 2010; McDiarmid and Altig, 1999). In the Neotropical genus *Allobates,* endotrophy has been reported in two species that are not closely related, *A. chalcopis* (Kaiser and Altig, 1994) and *A. nidicola* (Caldwell and Lima, 2003), thus suggesting independent evolution of endotrophy. Similarly, a striking pattern of evolution of larval development mode has been documented in the Malagasy *Gephyromantis* (Kaffenberger et al., 2012). A comparable pattern of intrageneric recurrent evolution of larval development is also known in *Adenomera* (*A. dyptix* and *A. thomei* being exotrophic in an otherwise endotrophic genus) (Fouquet et al., 2014). Nevertheless, to our knowledge, this is the first case of endotrophy/exotrophy evolution between species as closely related as in the *Anomaloglossus baeobatrachus* clade.

Delineating species is crucial for evaluating their threatened status (Bickford et al., 2007). Newly documented species can be formally described and their conservation status evaluated. Among the 21 species of *Anomaloglossus* currently listed in the IUCN Red List database, only six of them have been assessed for their conservation status. All the others are considered as "Data deficient". Nonetheless, some authors highlighted conservation urgency for these frogs (Fouquet et al., 2015; Kok et al., 2013). Some *Anomaloglossus* are already considered as threatened of extinction (*A. apiau*) when not probably already extinct (*A. tepequem*) (Fouquet et al., 2015). The perception that so many undescribed species are microendemics (*A.* sp. "Bakhuis", *A.* sp. "Itoupé") and that some nominal taxa are in fact also microendemics (*A. degranvillei*, *A. leopardus*) highlights the urge of evaluating the conservation status of these species.

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Chapter 4

Historical biogeography of *Anomaloglossus* (Amphibia: Anura: Aromobatidae) reveals single dispersal from the tepuis to the lowlands and multiple evolution of reproductive traits

Foreword to chapter 4

Parts of the *Material and methods* section have been published in the journal *Mitochondrial DNA Part B: Ressources* (Vacher et al., 2016). This article is reproduced in Appendix M.

4.1 Introduction

Tropical forests harbour the highest diversity of species on the planet (Dowle et al., 2013; Gaston, 2000; Hillebrand, 2004). Among them, those of the Neotropics (tropical America), notably Amazonia, are the richest for many groups (Antonelli and Sanmartín, 2011; Da Silva et al., 2005; Jenkins et al., 2013; Myers et al., 2000; Pyron and Wiens, 2013).

The origin of Amazonian diversity has puzzled biologists for almost two centuries (Bates, 1863; de Humboldt, 1820; Wallace, 1852), but still remains poorly understood. Actually, diversification in Amazonia was mostly explored in relation with adjacent biomes, and only few studies tackle *in situ* diversification (e.g., Antonelli et al. 2009; Brumfield et al. 2007; Elias et al. 2009; Fernandes et al. 2012; Gascon et al. 2000; Hughes and Eastwood 2006; Muñoz-Ortiz et al. 2015; Santos et al. 2009; Upham et al. 2013). Linnean and Wallacean shortfalls that affect the Amazonia biota are the two main reasons for such a lack of knowledge (Bush and Lovejoy, 2007; Hortal et al., 2015).

The Guiana Shield has been recognised as an Amazonian biogeographic region since the 19^{th} century, as Alfred Russell Wallace mentioned it as one of the four 'districts' of Amazonia (Wallace, 1852). Since this classification, it is now admitted that the highlands formations of the western Guiana Shield (the Pantepui region) are not included in the Amazonian biome (Vilhena and Antonelli, 2015), whereas the eastern Guiana Shield (EGS), a region bounded by the Amazon and Negro rivers to the south and the Pantepui and Orinoco basin to the north and that mostly comprises lowland rainforests, faunistically belongs to Amazonia. This region has been distinguished as a biogeographic area based on the distribution of birds (Naka, 2011; Naka et al., 2012), but our analysis based on frog distribution presented in Chapter 2 revealed a subdivision in three biogeographic areas within the EGS, with high endemism rates. The Guiana Shield have been quite stable during the Cenozoic, and therefore its fauna and flora were not subject to the strong modifications from the influence of harsh events that affected landscapes such as mountain uplift, or set up of large river basins as it was the case in the Andes or in central Amazonia (Hoorn et al., 2010; Lujan and Armbruster, 2011). Nevertheless, lineages that are endemic to the Guiana Shield indicate substential *in situ* diversification. The most striking radiation that originates from the Guiana Shield is the plant family Bromeliaceae, which certainly first diversified in the *tepuis* about 100 mya, and subsequently dispersed throughout adjacent areas of the Neotropics (Givnish et al., 2007, 2011). Other examples mostly concern endemic fauna or flora of the Pantepui region (Berry and Riina, 2005; Désamoré et al., 2010; Kok et al., 2012, 2016a,b; Rull, 2004, 2005; Salerno et al., 2012). Studies conducted on frogs also suggest that diversification events occurred in the eastern lowlands of the Guiana Shield (Fouquet et al., 2012). To our knowledge, diversification linked with exchanges between the western highlands and eastern lowlands of

the Guiana Shield has only been previously explored for Bromeliad plants, with primary diversification in the lowlands and subsequent colonisation of highlands in the family Rapataeceae for example (Givnish et al., 2000). Frogs are rather interesting models for phylogeographic and biogeographic studies, as they display low vagility compared to other terrestrial vertebrates commonly used as models such as birds, often display a philopatric behaviour, and all the more are constrained by climatic conditions (Zeisset and Beebee, 2008). Most of the Guiana Shield endemic species or groups of frogs are restricted to the *tepuis* (Barrio-Amorós, 2010; Faivovich et al., 2005, 2013; Heinicke et al., 2009; Kok et al., 2012, 2015, 2016a,b; Ron et al., 2016; Salerno et al., 2012). Only a few groups are distributed in both the *tepuis* and the eastern lowlands, among which the Guianan endemic Microhylidae genera *Otophryne* and *Synapturanus*, each comprising three nominal species. *Anomaloglossus* stands out from other endemic groups of frogs as it is the only one that displays a large diversity (26 currently described species) and is distributed throughout the entire Guiana Shield, with highland, middle-elevation and lowland species. Also, as we exposed in Chapter 3, the genus displays contrasting life-history traits, particularly in the lowland groups that harbour species that have exotrophic and phoretic tadpoles, others have endotrophic and phoretic tadpoles, and finally at least two species have endotrophic and nidicolous tadpoles. Our previous results suggest that acquisition of these characters occurred independently in the evolutionary history of the group. Therefore, the fact that this genus has diversified to such an extent both in the highlands and the lowlands and that this diversification may be linked with the acquisition of reproductive traits is very intriguing. Did *Anomaloglossus* originate from the *tepuis* as previous results suggest (see Chapter 3), and subsequently dispersed to the lowlands, and if so, can this dispersal be related to historical environment changes? Did the acquisition of less complex reproductive traits enabled the colonisation of new niches, thus enabling diversification in the lowlands?

In order to address these questions, we used a multilocus phylogenetic approach with mitogenomes obtained through New Generation Sequencing (NGS) and four nuclear loci for all species of *Anomolaglossus* which were available (1) investigate the phylogenetic relationships within *Anomaloglossus*, (2) infer the ancestral states of life history traits related to tadpole development, and (3) infer the historical biogeography of this group.

4.2 Material and methods

4.2.1 Collection of data in the field

The samples used in this study represent a subset of the samples used in Chapter 3. The field methods are described in section 3.2.1 of Chapter 3. The sample design for the present study was conceived so at least one sample of each candidate species retrieved in Chapter 3 would be included in the data pool, with a particular focus on the species from the lowlands of the eastern Guiana Shield (Fig. 4.1). Only *Anomaloglossus* sp. "Paru" was not included in the dataset as only a 400 bp fragment of the 16S rRNA was available for this lineage and we did not have additional tissue to perform DNA extraction for complementary sequencing. The non inclusion of this species did not impede the analysis given its position as the sister species of *A.* sp. "Brownsberg" is little ambiguous, and they share the same biogeographic area and most likely the same life history traits.

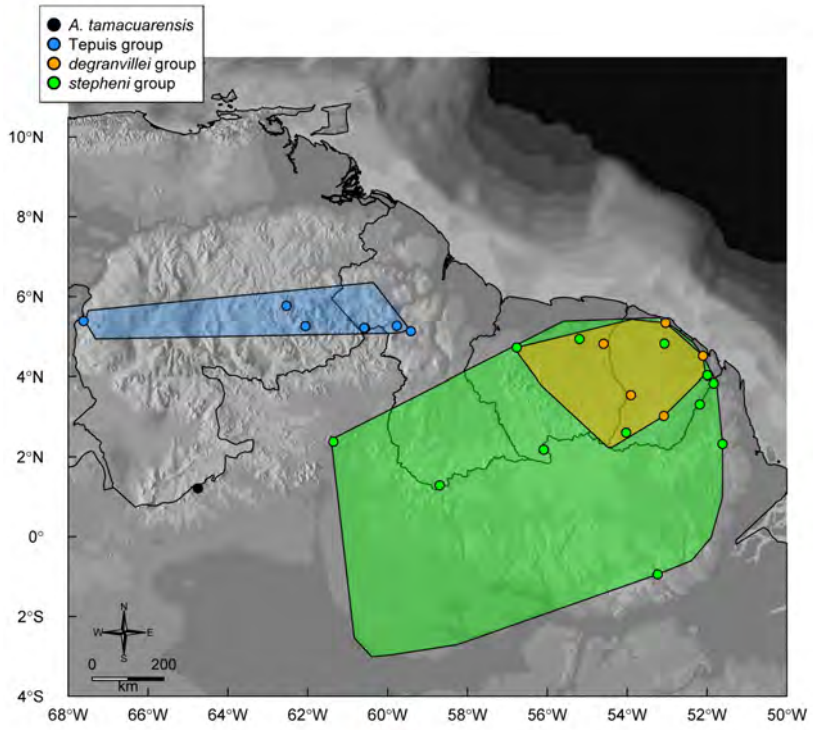

Figure 4.1 – Localisation of the *Anomaloglossus* samples used in this study. The ranges on the map correspond to the three main species group as defined in Chapter 3, plus *A. tamacuarensis*.

4.2.2 Molecular data

We extracted DNA from liver tissue of 27 samples of *Anomaloglossus* and 11 outgroups using the Wizard Genomic extraction protocol (Promega; Madison, WI, USA) (Supple-

mentary Table D.1). We sequenced sequenced the whole genome of these samples using a shotgun approach with an Illumina sequencer (Illumina, USA). Mitogenomes were assembled using the python-based organelle assembler Org.Asm (Coissac, 2016), coupled with a genome skimming approach implemented in Geneious (Besnard et al., 2014; Vacher et al., 2016). The mitochondrion genomes were annotated using MITOS web annotator (Bernt et al., 2013), and then corrected in Geneious v.9 (Kearse et al., 2012). We completed our dataset with 30 complete or incomplete mitogenomes of Hyloidea available in GenBank (Supplementary Table D.2). The same approach was used to retrieve the *28S* fragment. Additionally, we amplified and sequenced three protein-coding nuclear loci (tyrosinase - *TYR*; proopiomelanocortin C -*POMC*; and recombination activating gene exon 1 -*RAG1*). PCR conditions were similar as what we used for the 16S fragment (see Chapter 3 for details). We completed our dataset by adding 26 fragments of *TYR*, 34 fragments of *POMC*, and 34 fragments of *RAG1* sequences that were already available in GenBank. As the nuclear fragments were not always available for outgroup species that we selected in the mitogenomes dataset, we concatenated fragments from different species or from different genera when the monophyly of the targeted group was known from literature in order to complete as much as possible the matrix (Supplementary Table D.2). Novel sequences were deposited in GenBank and are listed in Supplementary Table D.2. Nucleotide sequences were aligned using MAFFT v.7 (Katoh and Standley, 2013) using default parameters (gap opening penalty = 1.53; gap extension penalty = 0.123; progressive method = FFT-NS-2) for the mitogenomes, the *28S* fragment, and the three nuDNA fragments. We discarded the control region of mitogenomes in the resulting alignment. Finally, we generated a concatenated dataset of the mitogenomes, *28S*, *TYR*, *POMC*, and *RAG1* using the program FasConcat v.1.0 (Kük & Meusemann 2010).

4.2.3 Phylogenetic analysis

A Maximum likelihood (ML) analysis was conducted with RAxML v.8.2.4 (Stamatakis, 2014) using the GTR+G model following a recommendation from the RAxML v.8.2.X manual (Stamatakis, 2015), applied on the mitogenome dataset and on the concatenated nuclear loci (*28S–TYR–POMC–RAG1*) dataset. Support of nodes was investigated with 1000 nonparametric bootstrap replicates using the fast bootstrapping algorithm. *Calyptocephalella gayi* and *Lechriodus melanopyga* (Myobatrachoidea) were used as outgroups

for the whole tree (Frost et al., 2006; Pyron, 2014; Pyron and Wiens, 2011; san Mauro et al., 2005).

4.2.4 Divergence time estimates

Divergence times were estimated using BEAST v2.4.1 (Bouckaert et al., 2014) in a Bayesian inference (BI) analysis using a relaxed uncorrelated clock (Drummond et al., 2006) on a concatenated dataset of mitogenomes with the four nuclear loci. We used the program PartitionFinder v1.1.1 (Lanfear et al., 2012) to select the best-fitting model of evolution for each partition using the Bayesian information criterion (BIC). A total of 42 partitions were treated:

- 1 nuclear rRNA
- 3 nuclear exons
- 2 mitochondrial rRNA
- 13 mitochondrial genes
- 22 mitochondrial tRNA
- 1 mitochondrial origin of replication

We used BEAUTi v2.4.1 to generate the input file containing prior parameters for the BI analysis. We applied a Birth-Death model for the tree prior. For divergence time estimation, we used secondary divergence time estimates that we implemented in BEAUTi with an uniform distribution for the following nodes: Myobatrachoidea, Hyloidea, Dendrobatoidea and Bufonidae (Table 4.1). A Markov Chain Monte Carlo (MCMC) was run for 50 million generations and sampled every 5,000 generations, resulting in 50,000 trees in the posterior distribution. A Maximum Clade Credibility (MCC) tree was obtained with Treeannotator v.2.4.1 (Rambaut and Drummond, 2012), applying a 50% burnin.

All computations were performed on EDB-Calc Cluster which uses a software developed by the Rocks(r) Cluster Group (San Diego Supercomputer Center, University of California, San Diego and its contributors), hosted by the laboratory “Evolution et Diversité Biologique” (EDB).

Node	Time of divergence	
	Calibration	Type of prior
Bufonidae	40 – 65 mya (Van Bocxlaer et al., 2009)	uniform prior (lower 40; upper 65; offset 0.0)
Dendrobatoidea	31 – 52 mya (Santos et al., 2009)	uniform prior (lower 31; upper 52; offset 0.0)
Hyloidea	65 – 100 mya (Marjanović and Laurin, 2007)	uniform prior (lower 65; upper 100; offset 0.0)
Myobatrachoidea	76 – 106 mya (Frazão et al., 2015)	uniform prior (lower 76; upper 106; offset 0.0)

Table 4.1 – Molecular clock scenarios used for the divergence time estimation, with the types and settings of priors.

4.2.5 Ancestral traits reconstruction

We used a stochastic character mapping approach to infer the ancestral states of two sets of traits associated with tadpole development: (1) exotrophic tadpoles vs. endotrophic tadpoles and (2) phoretic tadpoles vs. nidicolous tadpoles vs. phytotelm-dwelling tadpoles. We ran a first analysis by adding an "unknown" category for both states when we did not have any information on the reproductive mode or tadpole development mode. This applied to *A. leopardus*, *A.* sp. "Acari", and one individual assigned to *A. baeobatrachus* labelled MTR24258 from Amapá, Brazil. In a second analysis, we inferred the character states for these species as follow:

- *Anomaloglossus leopardus* was attributed exotrophy and phoresy based on its body size, its habitat and its call which are similar to other exotrophic and phoretic species (see Chapter 3);
- *A.* sp. "Acari" was attributed exotrophy;
- *Anomaloglossus baeobatrachus* MTR24258 was attributed endotrophy and nidicoly based on its body size that is similar to endotrophic and nidicolous species of its clade (see Chapter 3).

We applied a discrete trait reconstruction approach with a stochastic mutational mapping on phylogenies method (SIMMAP) with the packages *geiger* (Harmon et al., 2008)

and *phytools* (Revell, 2012) implemented in R (R Development Core Team, 2016). We created evolutionary model ER (single rate), SYM (symetrical), and ARD (All rates different) with the *fitDiscrete* function of the *geiger* package. We then selected the best model by calculating the delta of AICc[1] between models. We conducted two sets of analysis on the two sets of data with the function *make.simmap* of the package *phytools*. The first analysis used an empirical setting for the estimation of the parameter "Q". The second analysis used a MCMC setting for the estimation of the parameter "Q". For both analyses, we generated 1000 stochastically mapped trees. For an empirical estimate of "Q", we used symmetric transitions and default on the root. For an MCMC estimate of "Q", we set the sampling variances of MCMC ("vQ") to 0.01, and used a prior with $\alpha = \beta \times \text{ML(Q)}$, and with beta set to 2.

4.2.6 Biogeographic multimodel inference and ancestral range estimation

Previous to undertaking biogeographic analyses, we generated a subtree of the clade formed by Dendrobatoidea[2] from the global tree generated with BEAST with the "subtrees" function of the package *ape*. Then, in order to estimate the ancestral range for *Anomaloglossus* species and species groups, we defined nine biogeographic regions that correspond to the distribution of the different Dendrobatoidea groups that we used in the phylogenetic tree: Atlantic Forest (A), Chocó (A'), Southern Andes (B'), Northern Andes (B), Amazonia (C), Pantepui region (D), western EGS (E), southern EGS (F), eastern EGS (G), these last three areas being the ones recovered from Chapter 3. Model parameters and ancestral areas were reconstructed using an optimx routine in the package BioGeoBEARS (Matzke, 2013a) implemented in R (R Development Core Team, 2016) for six biogeographical models. The Dispersal Extinction Cladogenesis (DEC) model describes the temporal change in the range of a species, and distinguishes anagenetic change from cladogenetic change. It estimates two free parameters describing anagenesis: d, the rate of dispersal (range expansion) and e, the rate of extinction (range contraction) (Ree and Smith, 2008). We tested six different biogeographical models on our dataset:

- Dispersal Extinction Cladogenesis model (DEC) of Lagrange (Ree et al., 2005);

[1] AICc: sample-size corrected Akaike information criterion (AIC), used to reduce overfit of AIC.
[2] Clade containing Dendrobatidae and Aromobatidae, see Fig. H.1

- DEC+j, DEC with an additional "j" parameter accounting for founder event speciation during cladogenesis (Matzke, 2013b);
- DIVALIKE (DIVA), a likelihood version of the parsimony-based dispersal-vicariance analyses (Ronquist, 1997);
- DIVA+j, DIVALIKE with an additional "j" parameter accounting for founder event speciation during cladogenesis;
- BAYAREA-LIKE (BAYAREA), a likelihood version of the Bayesian inference of historical biogeography for discrete areas (Landis et al., 2013);
- BAYAREA+j, BAYAREA with an additional "j" parameter accounting for founder event speciation during cladogenesis.

The best fit of the models was assessed by comparing weighted Akaike's Information Criterion (AIC) scores, and we also evaluated likelihood values through a Likelihood Ration Test (LRT) (Matzke, 2013a).

We ran the analyses with the following adjustments of occurrence areas for the six different models: all the nodes above *Anomaloglossus* except the the two *Allobates* set to "Andes", given that the origin of Dendrobatoidea has been inferred to be in the Andes (Santos et al., 2009), and the sister clade of *Anomaloglossus* also originates from the Andes (Santos et al., 2009), and with *Anomaloglossus stepheni* set to "southern EGS" according to the results of Chapter 2 that indicate that this species originates from the southern EGS and expanded secondarily to the eastern EGS.

4.3 Results

4.3.1 Phylogenetic analysis an molecular dating

Dendrobatoidea (Dendrobatidae + Aromobatidae) was retrieved as the sister group of all other Hyloidea (Appendix Fig. G.1 and F.1). Within Dendrobatoidea, both families are retrieved as a well-supported clades, and within Aromobatidae, *Anomaloglossus* represent a very-well-supported clade with a crown age estimated at 29.6 mya (95 % CI 24.5–34.8 mya) (Appendix Fig. G.1 and F.1). *Anomaloglossus tamacuarensis*, a species from the southern Pantepui region (Fig. 4.1), is retrieved as the sister group of all other

Anomaloglossus. Three well-supported clades are emerging, corresponding to the three species group defined in Chapter 3: the "Tepuis" group, which encompasses all species that are found in the Pantepui region except *A. tamacuarensis*; the "*degranvillei*" group, which includes *A. surinamensis*, *A. degranvillei*, and all candidate species related to these two species; the "*stepheni*" group, which contains *A. stepheni*, *A. apiau*, *A. leopardus*, *A. baeobatrachus*, and all related candidate species (*A.* sp. "Acari", *A.* sp. "Bakhuis", *A.* sp. "Brownsberg", and *A.* sp. "Mitaraka"). The split between the "Tepuis" group and the lowlands clade formed by the "*degranvillei*" and "*stepheni*" groups is estimated in the late Oligocene, 25.5 mya (95 % CI 21.5–29.9 mya).

The "*degranvillei*" group comprises two clades that diverged in the mid-Miocene (13 mya, 95 % CI 9.9–16.6 mya), one containing *Anomaloglossus degranvillei*, *A.* sp. "north FG" and *A.* sp. "Itoupé" that started to diverge in the Pliocene (3.4 mya, 95 % CI 2.2–4.7 mya), and another regrouping the deep-conspecific lineages currently assigned to *A. surinamensis*, with a first split of the lineage from Bakhuis Mounts (Suriname) with the two other lineages that occurred in Miocene 8.7 mya (95 % CI 5.6–11.9 mya).

The estimate of the crown age of the "*stepheni*" group is 19.8 my (95 % CI 14.7–23.8). The sister position of *A. stepheni* to the whole group is retrieved with strong support (Fig. 4.5). *A.* sp. "Acari", *A.* sp. "Bakhuis", and *A.* sp. "Brownsberg" form a clade that diverged in the Miocene 9.9 mya (95 % CI 6.9–12.8 mya). The clade formed by *A.* sp. "Mitaraka", *A. leopardus* and *A. baeobatrachus* diverged more recently at the boundary between Miocene and Pliocene (5.3, 95 % CI 3.8–7 my). *Anomaloglossus baeobatrachus* is represented by two well-defined lineages that diverge in the late Pliocene (3.2 mya, 95 % CI 2.2–4.4 mya), and at least one of these lineages contains two distinct phenotypes (see Chapter 3 for details) (Fig. 4.2). The position of *A.* sp. "Mitaraka" as the sister species of (*A. leopardus* + *A. baeobatrachus*) is retrieved with high support (Fig. 4.2).

The mitogenome tree has an almost identical topology as the BI consensus tree for *Anomaloglossus*, the only differences being the position of *A. wothuja* as the sister group of the rest of the "Tepui" group in the mitogenome tree, whereas it is the sister species of *A. megacephalus* in the BI tree (Fig. 4.2 and H.1 in Appendix). Most of the relationships between *Anomaloglossus* species are not well-supported in the nuclear tree (Fig.

H.2 in Appendix). All the more, ML analysis of mitogenome-based only phylogeny and concatenated nuclear loci display some differences in their topology (Fig. H.1 and H.2 in Appendix). *Anomaloglossus leopardus* is the sister group of a clade formed by *A.* sp. "Mitaraka" and *A. baeobatrachus*, but the support is low. *Anomaloglossus* sp. "Acari" is not included in a clade with *A.* sp. "Bakhuis" and *A.* sp. "Brownsberg", but is retrieved as the sister group of the clade formed by *A. leopardus*, *A. baeobatrachus* and *A.* sp. "Mitaraka", with a rather high support (81 %).

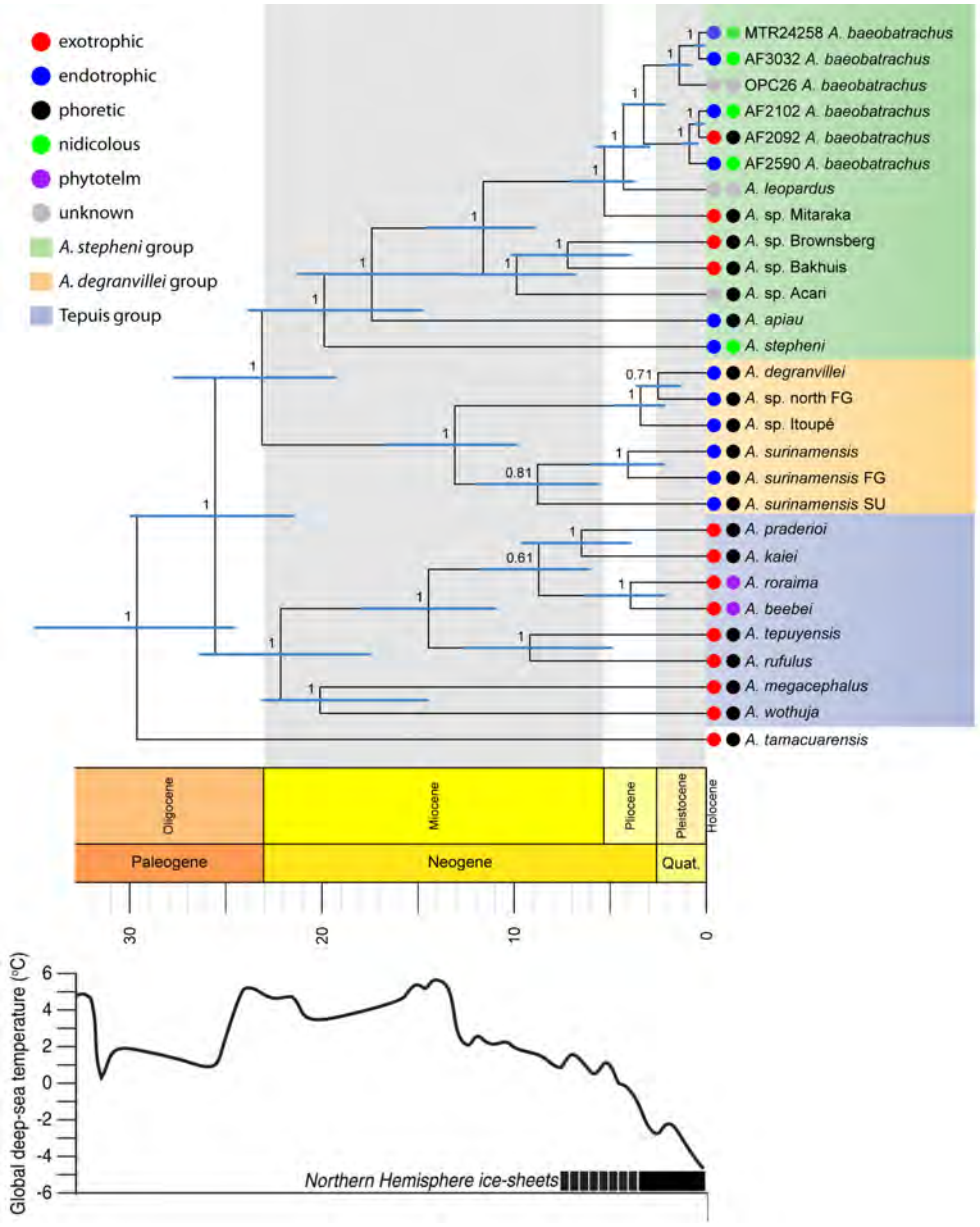

Figure 4.2 – Time-calibrated phylogeny of *Anomaloglossus* as recovered by BEAST with a secondary calibration. Posterior probabilities are indicated above nodes. Traits associated with tadpoles development are shown at the tip of the branches. The schematic timeline of paleoclimate is taken from Leite and Rogers (2013). Outgroups not shown. The numbers associated to the *A. baeobatrachus* specimens correspond to assigned field numbers. SU = Suriname; FG = French Guiana.

4.3.2 Inference of ancestral range

Our results indicate that every models with the addition of the "j" parameter confer more likelihood that the initial model (log-likelihood difference of 8 units for DEC, 6 units for DIVA and 24 units for BAYAREA, Table. 4.4). The three models, DEC+j, DIVA+j and BAYAREA+j have a log-likelihood ranging from 44 to 49, with DEC+j having the highest one (Table. 4.4).

The results of ancestral range inference are similar with these three models, the only notable difference being the ancestral range of the MRCA of *Anomaloglossus* with the rest of the Aromobatidae being inferred in the Tepuis with DIVA+j whereas it is inferred in the Andes with the two other models, with a strong probability under the BAYAREA model (Fig. 4.3). All ancestral range inferences within the *Anomaloglossus* clade are identical with the three models (Fig. 4.3 and Fig. I.1, I.2). The dispersal and extinction rates were estimated as <0.001 for the three models (Tab. 4.4). The "j" parameter was similar for the three models, at around 0.02 (Tab. 4.4). No vicariance event was inferred, and seven jump-dispersal events, including six within *Anomaloglossus* (Fig. 4.3). The rest of the events were sympatric range-copying events (Fig. 4.3). The proposed biogeographic scenario infers an Andean origin of the group under DEC+j and BAYAREA+j, with a first dispersal event to the tepuis 40 mya (Fig. 4.4A). After a first diversification event that correspond to the split of *A. tamacuarensis* from the rest of the group around 30 mya, a first dispersal event within *Anomaloglossus* occurred from the Tepuis to the eastern EGS around 25 mya (Fig. 4.4B). After the split between the "Tepuis" group and the EGS clade in the late Oligocene (∼25 mya), another dispersal event took place rapidly in the EGS in the late Oligocene–early Miocene, around 23 mya, which corresponds to the split between the two clades composing the "*stepheni*" and "*degranvillei*" groups (Fig. 4.4B). The range of the ancestor of these two clades was inferred to be in the eastern EGS, but these two groups have started to diversify allopatrically, the "*degranvillei*" group in the eastern EGS and the "*stepheni*" group in the southern EGS. The "*degranvillei*" group diversified in the eastern EGS but did not colonised the southern EGS. The history of the "*stepheni*" group is more complex. A first dispersal event from the the southern EGS to the eastern EGS occurred around 17 mya (Fig. 4.4C). A second dispersal event to the southern EGS was inferred around 9 mya and corresponds to the split between *A.*

sp. “Acari” with the species found nowadays in Suriname (*A.* sp. “Bakhuis” and *A.* sp. “Brownsberg”) (Fig. 4.4D).

Model	LnL	Parameter estimates			AIC	AIC model weight	Likelihood Ratio Test p-value
		d	e	j			
DEC	-52.29	0.001	1×10^{-12}	0	108.6	0%	5.8×10^{-5}
DEC+j	-44.21	0.0003	1×10^{-12}	0.019	94.44	100 %	
DIVA	-55.88	0.002	1×10^{-12}	0	115.8	0%	0.0003
DIVA+j	-49.36	0.001	1×10^{-12}	0.019	104.7	100 %	
BAYAREA	-73.56	0.001	0.02	0	151.1	0%	2.7×10^{-12}
BAYAREA+j	-49.1	0.0003	1×10^{-12}	0.019	104.2	100 %	

Table 4.2 – Comparison of biogeographic models with and without the j parameter. d: dispersal; e: extinction; j: jump-dispersal; LnL: log-likelihood; AIC: Akaike’s Information Criterion

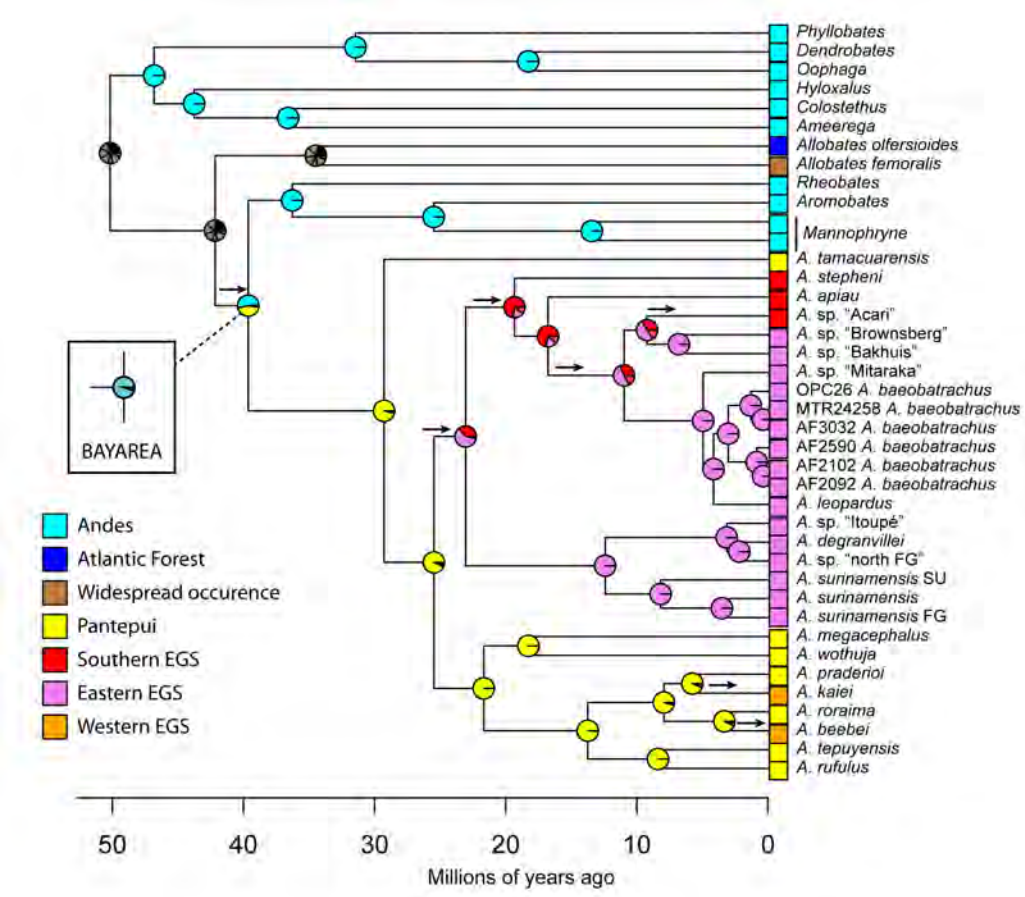

Figure 4.3 – Ancestral area reconstruction of Dendrobatoidea following the DEC+j model, with the range of all Dendrobatidae constrained to "Andes", and the range of *Anomaloglossus stepheni* constrained at southern EGS. Piecharts display the likelihood of each ancestral area; black arrows indicate dispersal events associated with cladogenesis. The tree topology is derived from the BEAST analysis. Colours represent area assignation for species at tips and most probable states at each node and stems. The states at nodes represent the most probable ancestral area before speciation, whereas states at stem represent the area of the descendant lineage right after speciation. The insert represent the estimate for this node under the BAYAREA model. EGS = Eastern Guiana Shield; FG = French Guiana; SU = Suriname.

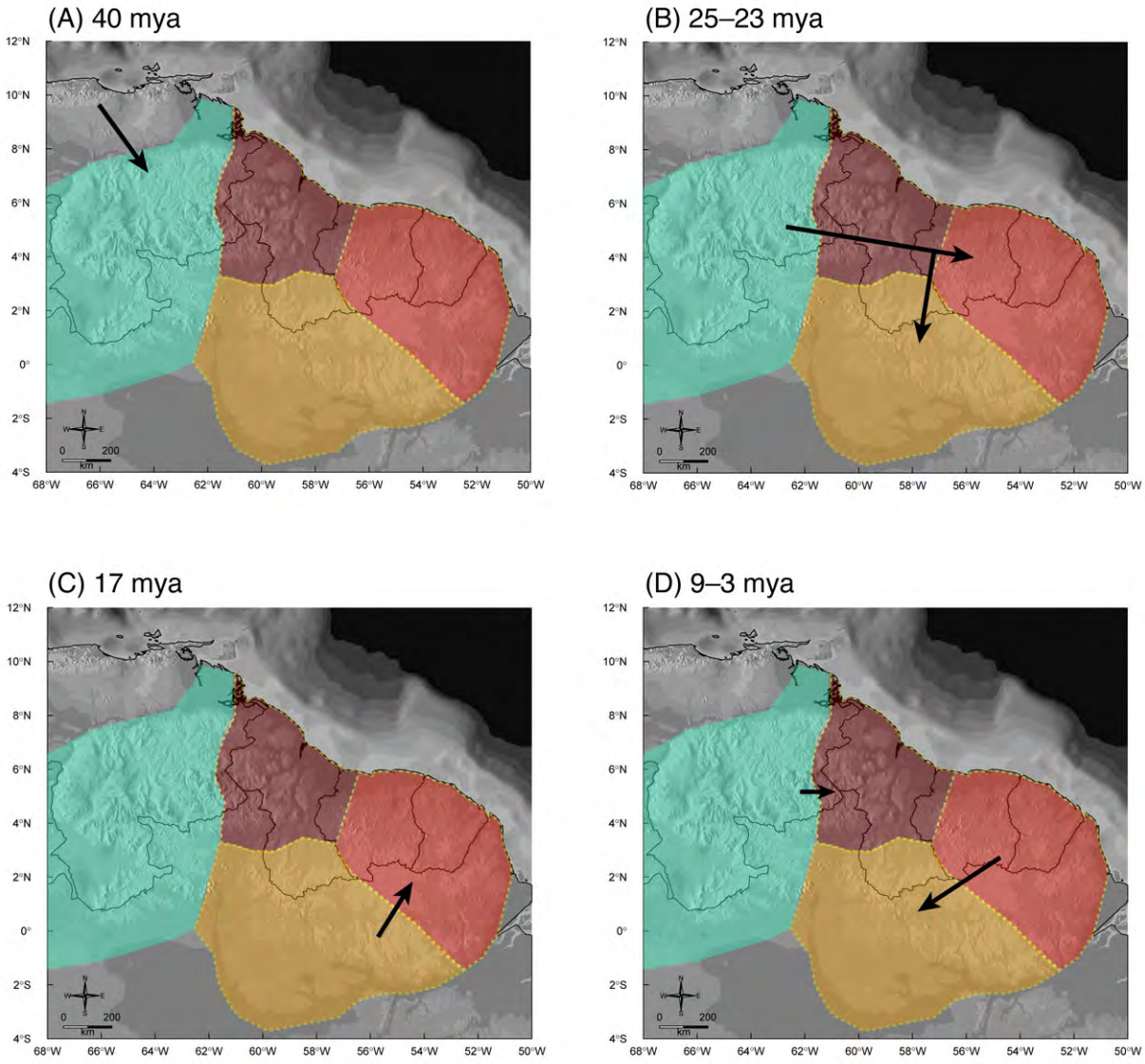

Figure 4.4 – Biogeographic scenario of the colonisation of the GS by *Anomaloglossus*. (A) First dispersal of the ancestor from the northern Andes to the Pantepui region, establishment of *Anomaloglossus* ; (B) Two dispersal events from the Tepuis to the north and southern zones of the EGS, corresponding to the divergence of the "*stepheni*" species group (south) and the "*degranvillei*" species group (north) ; (C) secondary dispersal of the "*stepheni*" species group from the south to the north of the EGS ; (D) Recent dispersal of some species of the "*stepheni*" group from the north to the south of the EGS, and dispersal of the phytotelm breeding species from the Tepuis to the western EGS. The yellow dashed lines materialise the boundaries between the three biogeographic regions of the EGS defined in Chapter 2 (cf. p. 50).

4.3.3 Ancestral character reconstruction

Both empirical and MCMC stochastic character mapping yielded similar results under the "ER" model when unknown characters traits were use in the database for *A. leopardus*, *A.* sp. "Acari" and MTR24258-*A. baeobatrachus*. The inference of ancestral characters differed for character 1 (exotrophy vs. endotrophy), with exotrophy being more likely for the MCRA of the "*stepheni*" and "*degranvillei*" groups, the node that correspond

to the MCRA of *A. stepheni* and the rest of the group, and the MCRA of *A. apiau* and the rest of the group (Fig. 4.5A and J.1A in Appendix). Also, when no unknown characters were inserted in the dataset, the MCRA of *A.* sp. "Mitaraka" with the clade formed by (*A. leopardus* and *A. baeobatrachus*) and the MRCA of *A. leopardus* and *A. baeobatrachus* were unambiguously exotrophic (Fig. 4.5B and J.1B in Appendix). The reconstruction of ancestral characters was almost exactly the same for character 2 (phoresy vs. nidicoly), with the exception of the MCRA of *A. leopardus* and *A. baeobatrachus* which was unambiguously inferred as phoretic with no unknown characters (Fig. 4.5B and J.1B in Appendix). We present in this chapter the results of the dataset that contains inferred characters for the three species *A.* sp. "Mitaraka", *A. leopardus*, and MTR24258-*A. baeobatrachus*.

Exotrophy vs. endrotrophy There were six possible changes of states, all of them occurred in our tree (Table 4.3). There were 5.8 changes on average between states along the tree.

Changes	1,2	2,1
x–>y	3.76	2.03

Table 4.3 – Average number of changes for traits associated with exotrophy and endotrophy. 1 = exotrophy; 2 = endotrophy

Phoresy vs. nidicoly vs. phytotelm breeders There was 12 possible changes of states, all of them occurred in our tree (Table 4.4). The average number of changes that involved phytotelm breeders with nidicoly or unknown states were always very low ($\leq$0.05) There was an average of 4.3 changes between states along the tree.

Changes	1,2	1,3	2,1	2,3	3,1	3,2
x–>y	2.04	1.09	1.07	0.00	0.07	0.02

Table 4.4 – Average number of changes for traits associated with phorey, nidicoly, and phytotelm breeders. 1 = phoresy; 2 = nidicoly; 3 = phytotelm breeders

Our results indicate that endotrophy appeared at least once, and is concomitant with the divergence of EGS species from Tepuis ones about 23 mya (Fig. 4.5A). Subsequently, there was at least two, or maybe three independent reversals toward exotrophy, one during the split of the clade formed by *A.* sp. "Acari", *A.* sp. "Brownsberg", and *A.* sp. "Bakhuis" about 11 mya, one during the divergence of *A.* sp. "Mitaraka" with the rest of the "*baeobatrachus*" clade, and more recently within the "*baeobatrachus*" group, certainly during the Pleistocene (fig. 4.5A). The ancestral trait of this character of the MCRA[3] of the (*A.* sp. "Acari"–*A.* sp. "Brownsberg"–*A.* sp. "Bakhuis") clade with the "*baeobatrachus*" clade could not be determined, as well as the state for the MCRA of *A.* sp. "Mitaraka" with the rest of the "*baeobatrachus*" group, as the likelihoods were almost 50 % for both nodes (Fig. 4.5A). Nidicoly appeared independently twice during the evolution history of *Anomaloglossus*, in the "*stepheni*" group (Fig. 4.5B). It appeared once during the divergence of *Anomaloglossus stepheni* during the Miocene, then appeared a second time during the diversification event of the *A. baeobatrachus* lineages in the late Pliocene (Fig. 4.5B). All nidicolous species are endotrophic, but acquisition of this character is decoupled from the acquisition of endotrophy as this last character occurred earlier in the evolutionary history of *Anomaloglossus*, as our results indicate the the MCRA of the "*degranvillei*" and the "*baeobatrachus*" group was certainly endotrophic and phoretic. Reversal to phoresy occurred only in *A. baeobatrachus*, and is associated with the independent acquisition of exotrophy within this species (Fig. 4.5).

[3]MCRA: most common recent ancestor

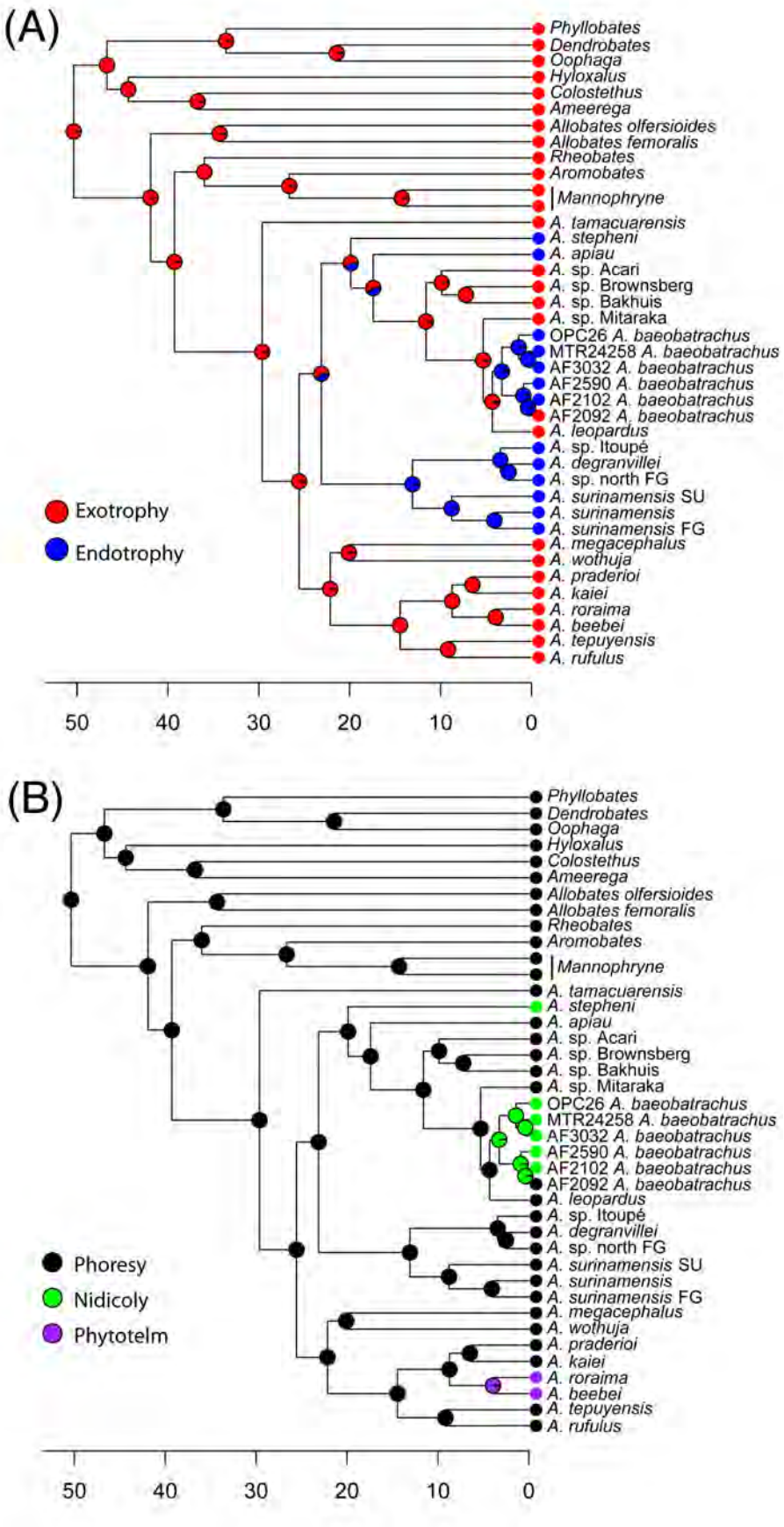

Figure 4.5 – Discrete trait reconstruction based on stochastic character mapping method with an empirical estimation of Q, for the dataset that includes no unknown states of characters. (A) exotrophy vs. endotrophy; (B) phoresy vs. nidicoly vs. phytotelm breeders.

4.4 Discussion

4.4.1 Biogeography of *Anomaloglossus*

Anomaloglossus is a unique example of radiation within an Amazonian subregion. To our knowledge, no other lineages have diversified in Amazonian lowlands to that extent in such a restricted spatial scale. Moreover, even at such a small scale, a clear biogeographic signal is detected.

Our results support the hypothesis that *Anomaloglossus* certainly originates from a dispersal event from the northern Andes to the the Pantepui region. This pattern has been observed for other Tepuis vertebrates such as birds (Bonaccorso et al., 2013; Mayr and Phelps, 1967; Sedano and Burns, 2010). However dispersal to the Tepuis occurred during the Pliocene in *Aulachorhynchus* toucanets (Bonaccorso et al., 2013) and tanagers (Sedano and Burns, 2010), a period that correspond to the end of the northern Andean uplift, when the northern Andes reached half of their current size, between 7 and 4 mya. Yet, our data indicate that *Anomaloglossus* certainly originated from a dispersal event from the northern Andes to the Pantepuis that dates from mid- to late Eocene (about 40 mya), a period when the northern Andes uplift has not begun, and only some isolated highland areas were present in this region (Hoorn et al., 2010). Data on global paleoclimate of earth indicate that the early Cenozoic Era was warm, with a peak during the early Eocene climatic optimum (∼50 mya). The Eocene climatic optimum was followed by a cooling period that lasted for about 17 my, with three periods of notable change, two in the Eocene between 50 and 48 mya and between 40 and 36 mya, and in the early Oligocene (35–34 mya) (Zachos et al., 2001). As the second cooling period of the Eocene correspond to the origin of *Anomaloglossus*, it is possible that cooler conditions triggered a range shift from higher to lower altitudes in some ectothermic organisms that were living in the few highland areas of the region during that period, thus enabling ancestors of *Anomaloglossus* to colonise the lowland areas of what constitutes nowadays the Llanos, between the northern Andes and the Pantepui region. Such a scenario would be in accordance with a long-distance dispersal from the northern Andes to the tepuis, even if some authors rejected it for small vertebrates (Leite et al., 2015; McDiarmid and Donnelly, 2005). Marine incursions that occurred in the Oligocene (∼33 mya), followed by the formation of the Pebas system in the

early Miocene, certainly fragmented terrestrial habitats between the northern Andes and the Guiana highlands over a long period of time (Hoorn et al., 2010), and could therefore have promoted allopatric speciation between *Anomaloglossus* and their Andean relatives such as what was suggested for Allophrynidae *vs.* Centrollenidae (Castroviejo-Fisher et al., 2014), *Stefania vs.* other Hemiphractids, or *Oreophrynella vs.* other Bufonids, and probably other groups whose centre of origin remains to be investigated, even though it is challenging at this time scale. A rapid warming-up of the climate that occurred in the late Paleogene could have triggered colonisation of higher elevations in the *Tepuis*, as this climatic event is concordant with the first diversification within *Anomaloglossus* (Fig. 4.2).

Concerning the timing of diversification in the Guiana Shield, it is to note that the crown age of the endemic frog genus *Stefania* has been inferred to the late Oligocene (around 26 mya) (Kok et al., 2016b), that the split between *Tepuihyla* and lowland *Osteocephalus* has been also been estimated to late Oligocene, at 24.7 mya (Salerno et al., 2012), and that the crown age of *Otophryne* has been inferred during the Oligocene (∼29-30 mya) (de Sa et al., 2012). Our estimates for the crown-age of *Anomaloglossus* are concordant with this period (∼29 mya (95 % confidence interval 34.8–24.5, Fig. 4.2). Therefore, it is possible that the events that triggered the first diversification events within *Anomaloglossus* were similar to the ones for the other groups, which is likely due to habitat shift or vertical displacement that were generated by late Oligocene warming around 25 mya (Fig. 4.2) (Salerno et al., 2012).

During the Miocene, the uplift of the northern Andes continued and generated profound changes in climate regime in Amazonia, as well as the establishment of the Amazon basin from west to east (Hoorn et al., 2010). Warmer climates during the first half of the Miocene could have influence a range shift towards higher elevation on the tepuis, thus triggering speciation in this group, which is in accordance with our results (Fig. 4.2), even though our sampling of tepuis *Anomaloglossus* is lacking a lot of species (our dataset includes 9 out of 20 species). It is not clear though what triggered a dispersal from the tepuis to the eastern lowlands (EGS). Another documented example of colonisation of the lowlands from the Tepuis would be the frog genus *Otophryne*, in which the high-elevation dwelling species *0. steyermarki* is the sister group to the two other the species

that occur at mid- and low elevations (de Sa et al., 2012). This dispersal event actually occurred during the late Oligocene warming, an episode that was quite rapid, estimated to occur between 27 and 25 mya, followed by a long period of warm climate until the mid-Miocene (∼15 mya). The dispersal from the *tepuis* to the lowlands is concomitant with the emergence of a new reproductive mode (endotrophic tadpoles) thus enabling populations to become independent of aquatic habitats and shift their ranges to warmer and more humid habitats of the lowland forests. Such a hypothesis is in accordance with our results, as the MRCA of the whole EGS clade was likely to be endotrophic (Fig. 4.5).

Dispersal to the eastern EGS from southern EGS occurred between 23–19 mya, during a stable warm period of the Miocene. An endotrophic ancestor certainly benefited from such climatic conditions, and consequently could have expanded its range in the eastern EGS by colonising *terra firme* habitats in large patches of lowland forests. A reversal from endotrophy to exotrophy in the "*stepheni*" group certainly occurred around 13 mya (Fig. J.1 in Appendix), a period that corresponds to a cooling of the climate during mid-Miocene (Hoorn, 1994). During this period, *terra firme* lowland forest may have been fragmented into patches, or at least precipitation regimes may have been disturbed, causing species ranges to become fragmented (Pons and De Franceschi, 2007). If such landscape conditions were indeed present, it is possible that populations could have survived in such environments for long periods of time, thus leading to allopatric speciation. Dryer conditions would have favoured the exotrophic and phoretic phenotype as it is likely less dependent on atmospheric humidity than the endotrophic form. Endotrophic and nidicolous phenotype was certainly confined to a restricted range, but could colonise again rapidly large areas during more recent warmer and humid episodes. This certainly explain why species that share the same phenotype display allopatric distributions nowadays, whereas *A. stepheni* is sympatric with exotrophic *A.* sp. "Brownsberg", *A.* sp. "Mitaraka", *A. leopardus*, and probably with *A.* sp. "Bakhuis" and *A.* sp. "Acari", while endotrophic *A. baeobatrachus* is sympatric with exotrophic *A. baeobatrachus* and *A.* sp. "Mitaraka". It would therefore be necessary to provide fine distribution ranges for each species occurring in the region, and to design a fine geographic division of the eastern EGS.

4.4.2 Pattern and timing of character acquisition

To our knowledge, the pattern of trait distribution within *Anomaloglossus* represents the only case of independent shift of characters among closely-related species within the same genus of frogs. Also, our data suggest that evolution of exotrophy from endotrophy occurred at least twice within the genus (Fig. 4.5). Such a pattern is unexpected as reversal from endotrophy to exotrophy is very rare in frogs (Gomez-Mestre et al., 2012).

Acquisition of endotrophy seems to be associated with the first divergence event between tepuis species and lowland clades as our results clearly indicate independent and repeated acquisition of exotrophy and nidicoly within *Anomaloglossus* (Fig. 4.5).

The case of *Anomaloglossus baeobatrachus* is more complex. As we saw in Chapter 3, it is highly probable that our tree does not reflect the actual relationships between species, and that actually exotrophic and phoretic *A. baeobatrachus* and endotrophic and nidicolous *A. baeobatrachus* are two different species that are not distinguishable with the markers that we used for this study. This taxonomical uncertainty blurs the interpretation of the results. Therefore, if we consider that *A. leopardus* is exotrophic and phoretic, then the ancestral state of the MRCA of *A. leopardus* and *A. baeobatrachus* would unambiguously be exotrophic and phoretic. Also, in a scenario where both phenotypes of *A. baeobatrachus* were considered as different species and *A. baeobatrachus* was the endotrophic and nidicolous one, endotrophy associated with nidicoly would only have occurred twice independently in the "*stepheni*" group, once in *A. stepheni*, and once in *A. baeobatrachus*, and the ancestral state of the clade composed by *A.* sp. "Mitaraka", *A. leopardus* and *A. baeobatrachus* would certainly be exotrophic and phoretic. In such a scenario, the re-acquisition of endotrophy and nidicoly in *A. baeobatrachus* would have been triggered by an event that occurred at a more recent times than for *A. stepheni*, around 5 mya, which correspond to early Pliocene.

Evolution from a direct developer[4] MRCA to aquatic tadpoles has already been demonstrated in the Neotropical family Hemiphractidae, with at least two cases of independent evolution from direct development to free-living tadpoles (Castroviejo-Fisher et al., 2015;

[4]Direct-developer is used here as opposed to free-living tadpole, not *sensu* Altig and Johnston (1989)

Wiens et al., 2007). The pattern of trait evolution observed in the "*stepheni*" group represents the second known case of evolution from direct developer to free-living tadpoles, with at least two independent events, and maybe four. Getting a clearer view of this pattern of trait evolution within the "*stepheni*" group would require to complete the gaps in knowledge on reproductive traits for some species (species marked "unknown" in Figs. 4.2 and J.1 in Appendix), and also to resolve the taxonomic riddle of *A. baeobatrachus*. Another example of contrasting life-history traits within a group is found in Madagascar, as the genus *Gephyromantis* also countains nidicolous species vs. free-living tadpoles ones, and exotrophic vs. endotrophic species, but frogs possessing different traits actually form clades (Kaffenberger et al., 2012).

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Chapter 5

Conclusion

At the brink of the sixth mass extinction of biodiversity, we are still struggling to estimate the actual magnitude of diversity in the tropics. The present work brings new insights at the actual species richness of anuran amphibians in Amazonia, and highlights the gap that needs to be filled to understand the foundations of tropical ecosystems. Results presented in Chapter 2 indicate that the number of Amazonian frogs is vastly underestimated, and that regional endemism could be as high as 82 % in the eastern Guiana Shield, instead of 33 % as currently suggested by the IUCN. This large difference shed doubts on conclusions regarding Amazonia presented in analyses based on IUCN data. The threats faced by Amazonian amphibians such as impact caused by climate change could actually be far more serious than what is currently estimated. Also, my results hint concern on the conservation of Amazonian amphibians as entire subregions of southeastern Amazonia that might harbour similar degrees of endemism are currently vanishing, mainly due to deforestation.

The gap in our understanding of the Amazonian diversity is so large that it has also hampered tackling questions about the processes of diversification within that region. Using an unprecedented database, I managed to unravel a yet undocumented pattern of diversity within the Guiana Shield that does not seem to have emerge from current landscape features, but probably from past climate and current heterogeneity (Chapter 2). This pattern should be investigated in other taxa, and such an approach should be extended to the whole Amazonia. Actually, collaborative projects are underway. Determining the inherent structure of Amazonian diversity will allow formulating strong

hypotheses for several fields of biology, notably biogeography. The description of the pattern of diversity at the scale of the entire anuran fauna of Amazonia also opens a window toward the investigation of the evolutionary processes of the major clades of frogs. Once species boundaries and distributions are better understood, comparative analyses can be made on solid bases and sampling design can be optimised. In that way, I hope that the present work will foster further research on the diversification of Amazonian anurans.

In fact, I contributed to this feat by exploring species delineation in *Anomaloglossus* (Chapter 3). This genus is peculiar in the sense that it is the only group of frog that has diversified both in the Guiana Shield highlands and lowlands. The analyses presented in Chapter 3 revealed that like in most groups, the diversity existing in that genus was largely underestimated. The integrative analyses that I applied confirmed the delineation based on a single barcode, but also revealed contradictions. Indeed, even though morphological and bioacoustical data were available, I could not to confirm the specific status of several candidate species retrieved with barcodes in the "*degranvillei*" group. Similarly, two putative different species of the "*stepheni*" group were not retrieved as candidate species in the delineation analysis based on barcodes. The contrast in the phenotypic evolution in the "*degranvillei*" group is striking and may have resulted from interspecific competition, an hypothesis that would require further investigations. The present work also enabled to unravel an incredible variety of reproductive traits in the "*stepheni*" group. The evolution of larval development in that group is puzzling and should be investigated in much more details, especially in the *baeobatrachus* clade. Even though I formulated scenarios to explain such a remarkable pattern, I could not test them with the set of data that were produced and available. Further work would be necessary to tackle this question through a population genetics approach. An additional spectacular characteristic of that genus lies in the spatio-temporal features of its diversification (Chapter 4). The results that I presented revealed that this clade is rather ancient (∼30 my) and has apparently dispersed to the lowlands of the eastern Guiana Shield (EGS) only once about 25 mya, leading to the 12 currently known species. This probably represents the most striking radiation in such a small area in Amazonia. Moreover, despite this geographic confinement, a biogeographic signal is very clear even within the EGS. The evolutionary history of *Anomaloglossus* reveals that an extent forest existed over the last 20 my, and has probably been fragmented but diversification in that group also seems linked to reproductive traits shifts coupled

with climate fluctuation.

In conclusion, I hope that the present work will prompt similar studies elsewhere in Amazonia, as well as in the Guiana Shield on other groups. Understanding the diversity of Amazonia is crucial and urgent for its conservation, and I hope that I have contributed to improve the knowledge on Amazonian diversification and hopefully that my results will be used in the future for its conservation.

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Appendix A

Anomaloglossus samples

Table S1. List of specimens used in the present study for each loci, with GenBank accession numbers. New sequences are indicated in bold. Lat: Latitude; Lon: Longitude; AM: Amazonas, Brazil; AP: Amapá, Brazil; BA: Bahia, Brazil; CO: Colombia; EC: Ecuador; FG: French Guiana; GY: Guyana; PA: Pará, Brazil; RR: Roraima, Brazil; SR: Suriname; VE: Venezuela.

Field number	Species	Lat	Lon	Locality	Country/ Province	*16S*	*TYR*	*POMC*	*RAG1*
1339	*Anomaloglossus roraima*	5.2332	-60.7425	Mazaruni-Potoro, Mt. Roraima	GY	DQ502260	—	—	—
214	*Anomaloglossus rufulus*	5.25951	-62.0595	Churi-tepui	VE	—	—	**KY549491**	**KY549447**
216	*Anomaloglossus tepuyensis*	5.7690	-62.5340	Auyan-tepui	VE	—	—	**KY549492**	**KY549448**
929NZCS	*Anomaloglossus surinamensis*	3.8048	-56.1539	Tafelberg	SR	**KY510025**	—	—	—
937NZCS	*Anomaloglossus surinamensis*	3.8048	-56.1539	Tafelberg	SR	**KY510026**	—	—	—
954NZCS	*Anomaloglossus surinamensis*	3.8048	-56.1539	Tafelberg	SR	**KY510027**	—	—	—
AF0022	*Anomaloglossus baeobatrachus*	3.6255	-53.2072	Saül	FG	EU201070	—	—	—
AF0023	*Anomaloglossus baeobatrachus*	3.6255	-53.2072	Saül	FG	EU201070	—	—	—
AF0026	*Anomaloglossus surinamensis*	3.6255	-53.2072	Saül	FG	JN691083	—	—	—
AF0038	*Anomaloglossus degranvillei*	3.6000	-53.2833	Saül	FG	EU201081	—	—	—
AF0039	*Anomaloglossus degranvillei*	3.6000	-53.2833	Saül	FG	JN691103	—	—	—
AF0055	*Anomaloglossus baeobatrachus*	5.066667	-53.0500	Petit Saut	FG	JN690995	—	—	—
AF0093	*Anomaloglossus stepheni*	5.1833	-55.6166	Road to Apura	SR	JN691116	—	—	—
AF0094	*Anomaloglossus stepheni*	5.1833	-55.6166	Road to Apura	SR	JN691113	—	—	—
AF0095	*Anomaloglossus stepheni*	5.1833	-55.6166	Road to Apura	SR	JN691114	—	—	—
AF0096	*Anomaloglossus stepheni*	5.1833	-55.6166	Road to Apura	SR	JN691115	—	—	—
AF0122	*Anomaloglossus baeobatrachus*	4.9365	-55.1948	Brownsberg	SR	JN691046	—	—	—
AF0138	*Anomaloglossus surinamensis*	4.9365	-55.1948	Brownsberg	SR	JN691096	—	—	—
AF0139	*Anomaloglossus surinamensis*	4.9365	-55.1948	Brownsberg	SR	JN691097	—	—	—
AF0143	*Anomaloglossus surinamensis*	4.9365	-55.1948	Brownsberg	SR	EU201078	—	—	—
AF0149	*Anomaloglossus* sp. “Brownsberg”	4.9365	-55.1948	Brownsberg	SR	JN691042	—	—	—

AF0151	*Anomaloglossus* sp. “Brownsberg”	4.9365	-55.1948	Brownsberg	SR	JN691043	—	—	—
AF0152	*Anomaloglossus surinamensis*	4.9365	-55.1948	Brownsberg	SR	JN691098	—	—	—
AF0156	*Anomaloglossus* sp. “Brownsberg”	4.9365	-55.1948	Brownsberg	SR	JN691044	—	—	—
AF0188	*Anomaloglossus* sp. “Brownsberg”	4.9365	-55.1948	Brownsberg	SR	JN691045	—	—	—
AF0207	*Anomaloglossus baeobatrachus*	5.3833	-53.6500	Angoulême	FG	JN690987	—	—	—
AF0208	*Anomaloglossus baeobatrachus*	5.3833	-53.6500	Angoulême	FG	JN690977	—	—	—
AF0210	*Anomaloglossus baeobatrachus*	5.3833	-53.6500	Angoulême	FG	JN690978	—	—	—
AF0213	*Anomaloglossus surinamensis*	5.3833	-53.6500	Angoulême	FG	JN691048	—	—	—
AF0214	*Anomaloglossus baeobatrachus*	5.3833	-53.6500	Angoulême	FG	JN690975	—	—	—
AF0215	*Anomaloglossus surinamensis*	5.3833	-53.6500	Angoulême	FG	JN691050	—	—	—
AF0216	*Anomaloglossus baeobatrachus*	5.3833	-53.6500	Angoulême	FG	JN690988	—	—	—
AF0217	*Anomaloglossus baeobatrachus*	5.3833	-53.6500	Angoulême	FG	JN690976	—	—	—
AF0218	*Anomaloglossus surinamensis*	5.3833	-53.6500	Angoulême	FG	JN691051	—	—	—
AF0220	*Anomaloglossus surinamensis*	5.3833	-53.6500	Angoulême	FG	JN691049	—	—	—
AF0225	*Anomaloglossus surinamensis*	5.3833	-53.6500	Angoulême	FG	JN691052	—	—	—
AF0243	*Anomaloglossus baeobatrachus*	4.0833	-52.6833	Nouragues	FG	JN690989	—	—	—
AF0244	*Anomaloglossus baeobatrachus*	4.0833	-52.6833	Nouragues	FG	JN690992	—	—	—
AF0245	*Anomaloglossus surinamensis*	4.0833	-52.6833	Nouragues	FG	JN691053	—	—	—
AF0250	*Anomaloglossus surinamensis*	4.0833	-52.6833	Nouragues	FG	JN691062	—	—	—
AF0252	*Anomaloglossus baeobatrachus*	4.0833	-52.6833	Nouragues	FG	JN690986	—	—	—
AF0254	*Anomaloglossus baeobatrachus*	4.0833	-52.6833	Nouragues	FG	JN690985	—	—	—
AF0258	*Anomaloglossus surinamensis*	4.0833	-52.6833	Nouragues	FG	JN691054	—	—	—
AF0259	*Anomaloglossus surinamensis*	4.0833	-52.6833	Nouragues	FG	JN691055	—	—	—
AF0260	*Anomaloglossus surinamensis*	4.0833	-52.6833	Nouragues	FG	JN691056	—	—	—
AF0271	*Anomaloglossus baeobatrachus*	4.0916	-52.7000	Nouragues	FG	JN690991	—	—	—
AF0285	*Anomaloglossus surinamensis*	5.0666	-52.7166	Montagne des Singes	FG	JN691057	—	—	—
AF0286	*Anomaloglossus surinamensis*	5.0666	-52.7166	Montagne des Singes	FG	JN691058	—	—	—
AF0287	*Anomaloglossus surinamensis*	5.0666	-52.7166	Montagne des Singes	FG	JN691059	—	—	—
AF0288	*Anomaloglossus surinamensis*	5.0666	-52.7166	Montagne des Singes	FG	JN691060	—	—	—

AF0289	*Anomaloglossus baeobatrachus*	5.0666	-52.7166	Montagne des Singes	FG	JN690979	—	—	—
AF0291	*Anomaloglossus baeobatrachus*	4.5480	-52.1519	Kaw	FG	JN690980	—	—	—
AF0292	*Anomaloglossus* sp. “north FG”	4.5161	-52.1005	Montagne de Kaw	FG	JN691107	—	—	—
AF0293	*Anomaloglossus* sp. “north FG”	4.5161	-52.1005	Montagne de Kaw	FG	JN691108	—	—	—
AF0294	*Anomaloglossus* sp. “north FG”	4.5161	-52.1005	Montagne de Kaw	FG	JN691109	—	—	—
AF0296	*Anomaloglossus baeobatrachus*	4.5161	-52.1005	Montagne de Kaw	FG	JN690981	—	—	—
AF0536	*Anomaloglossus baeobatrachus*	4.1959	-52.1490	Savane Virginie	FG	**KY510028**	—	—	—
AF0537	*Anomaloglossus baeobatrachus*	4.1959	-52.1490	Savane Virginie	FG	**KY510029**	—	—	—
AF0539	*Anomaloglossus baeobatrachus*	4.1959	-52.1490	Savane Virginie	FG	**KY510030**	—	—	—
AF0548	*Anomaloglossus baeobatrachus*	4.1959	-52.1490	Savane Virginie	FG	**KY510031**	—	—	—
AF0573	*Anomaloglossus baeobatrachus*	3.0250	-53.0800	Mont Itoupé	FG	**KY510032**	—	—	—
AF0581	*Anomaloglossus baeobatrachus*	3.0250	-53.0800	Mont Itoupé	FG	**KY510033**	—	—	—
AF0584	*Anomaloglossus surinamensis*	5.3415	-53.0388	Piste Saint-Elie	FG	**KY510034**	—	—	—
AF0585	*Anomaloglossus surinamensis*	5.3415	-53.0388	Piste Saint-Elie	FG	**KY510035**	—	—	—
AF0615	*Anomaloglossus baeobatrachus*	3.0250	-53.0800	Mont Itoupé	FG	**KY510036**	—	—	—
AF0617	*Anomaloglossus baeobatrachus*	3.0250	-53.0800	Mont Itoupé	FG	**KY510037**	—	—	—
AF0618	*Anomaloglossus baeobatrachus*	3.0250	-53.0800	Mont Itoupé	FG	**KY510038**	—	—	—
AF0619	*Anomaloglossus baeobatrachus*	3.0250	-53.0800	Mont Itoupé	FG	**KY510039**	—	—	—
AF0621	*Anomaloglossus surinamensis*	3.0250	-53.0800	Mont Itoupé	FG	**KY510040**	**KY549532**	—	**KY549449**
AF0657	*Anomaloglossus baeobatrachus*	5.0248	-52.7391	Couy Kourou	FG	**KY510041**	—	—	—
AF0658	*Anomaloglossus baeobatrachus*	5.0248	-52.7391	Couy Kourou	FG	**KY510042**	—	—	—
AF0688	*Anomaloglossus baeobatrachus*	5.2754	-52.9236	Paracou	FG	**KY510043**	—	—	—
AF0718	*Anomaloglossus baeobatrachus*	5.2754	-52.9236	Paracou	FG	**KY510044**	—	—	—
AF0808	*Anomaloglossus baeobatrachus*	4.1959	-52.1490	Savane Virginie	FG	**KY510045**	—	—	—
AF0821	*Anomaloglossus baeobatrachus*	5.0312	-54.0877	Crique Voltaire	FG	**KY510046**	—	—	—
AF0822	*Anomaloglossus baeobatrachus*	5.0312	-54.0877	Crique Voltaire	FG	**KY510047**	—	—	—
AF0825	*Anomaloglossus surinamensis*	5.0312	-54.0877	Crique Voltaire	FG	**KY510048**	—	—	—
AF0857	*Anomaloglossus surinamensis*	5.0974	-53.0506	Chutes Grégoire	FG	**KY510049**	—	—	—
AF0865	*Anomaloglossus baeobatrachus*	5.097472	-53.0506	Pic Coudreau	FG	**KY510050**	—	—	—

AF0879	*Anomaloglossus baeobatrachus*	4.1959	-52.1490	Savane Virginie	FG	**KY510051**	—	—	—
AF0901	*Anomaloglossus surinamensis*	2.2534	-54.3534	Pic Coudreau Sud	FG	**KY510052**	—	—	—
AF0902	*Anomaloglossus surinamensis*	2.2534	-54.3534	Pic Coudreau Sud	FG	**KY510053**	—	—	—
AF0903	*Anomaloglossus baeobatrachus*	2.2534	-54.3534	Pic Coudreau Sud	FG	**KY510054**	—	—	—
AF0904	*Anomaloglossus* sp. “Mitaraka”	2.2534	-54.3534	Pic Coudreau Sud	FG	**KY510055**	—	—	—
AF0905	*Anomaloglossus* sp. “Mitaraka”	2.2534	-54.3534	Pic Coudreau Sud	FG	**KY510056**	—	—	—
AF0932	*Anomaloglossus* sp. “north FG”	4.5161	-52.1005	Crique Patawa	FG	**KY510057**	—	—	—
AF0933	*Anomaloglossus* sp. “north FG”	4.5161	-52.1005	Crique Patawa	FG	**KY510058**	—	—	—
AF0952	*Anomaloglossus* sp. “north FG”	4.0639	-52.0416	Route nationale 2	FG	**KY510059**	—	—	—
AF0953	*Anomaloglossus* sp. “north FG”	4.0639	-52.0416	Route nationale 2	FG	**KY510060**	**KY549533**	**KY549493**	**KY549450**
AF0955	*Anomaloglossus baeobatrachus*	4.0639	-52.0416	Route nationale 2	FG	**KY510061**	—	—	—
AF0977	*Anomaloglossus baeobatrachus*	3.6359	-53.2725	Galbao	FG	**KY510062**	—	—	—
AF0985	*Anomaloglossus baeobatrachus*	3.6359	-53.2725	Galbao	FG	**KY510063**	—	—	—
AF1045	*Anomaloglossus surinamensis*	3.6359	-53.2725	Saül	FG	**KY510064**	—	—	—
AF1113	*Anomaloglossus surinamensis*	5.0994	-53.8005	Fleuve Mana	FG	**KY510065**	—	—	—
AF1114	*Anomaloglossus surinamensis*	5.0994	-53.8005	Fleuve Mana	FG	**KY510066**	—	**KY549494**	**KY549451**
AF1147	*Anomaloglossus surinamensis*	4.6025	-53.4143	Trinité	FG	**KY510067**	—	—	—
AF1332	*Anomaloglossus* sp. “north FG”	3.8974	-52.5835	Saut Grand Machicou	FG	**KY510068**	—	—	—
AF1600	*Anomaloglossus surinamensis*	4.0847	-52.6806	Nouragues	FG	**KY510069**	—	—	—
AF1605	*Anomaloglossus baeobatrachus*	3.5731	-53.1985	Saül	FG	**KY510070**	—	—	—
AF1629	*Anomaloglossus baeobatrachus*	3.5731	-53.1985	Saül	FG	**KY510071**	—	—	—
AF1689	*Anomaloglossus baeobatrachus*	3.5631	-53.1908	Saül	FG	**KY510072**	—	—	—
AF1746	*Anomaloglossus surinamensis*	4.9957	-54.0925	Crique Voltaire	FG	**KY510073**	—	—	—
AF1747	*Anomaloglossus surinamensis*	4.9957	-54.0925	Crique Voltaire	FG	**KY510074**	—	—	—
AF1773	*Anomaloglossus surinamensis*	5.2492	-54.206	Apatou	FG	**KY510075**	—	—	—
AF1784	*Anomaloglossus baeobatrachus*	5.2273	-54.1665	Apatou	FG	**KY510076**	—	—	—
AF1838	*Anomaloglossus baeobatrachus*	4.1058	-52.0508	Route nationale 2	FG	**KY510077**	—	—	—
AF1839	*Anomaloglossus baeobatrachus*	4.1058	-52.0509	Route nationale 2	FG	**KY510078**	—	—	—
AF1840	*Anomaloglossus baeobatrachus*	4.1058	-52.0509	Route nationale 2	FG	**KY510079**	—	—	—

AF1841	*Anomaloglossus baeobatrachus*	4.1058	-52.0509	Route nationale 2	FG	**KY510080**	—	—	—
AF1842	*Anomaloglossus baeobatrachus*	4.1058	-52.0507	Route nationale 2	FG	**KY510081**	—	—	—
AF1843	*Anomaloglossus baeobatrachus*	4.1053	-52.0493	Route nationale 2	FG	**KY510082**	—	—	—
AF1844	*Anomaloglossus baeobatrachus*	4.1053	-52.0493	Route nationale 2	FG	**KY510083**	—	—	—
AF1845	*Anomaloglossus baeobatrachus*	4.1056	-52.0493	Route nationale 2	FG	**KY510084**	—	—	—
AF1846	*Anomaloglossus baeobatrachus*	4.1056	-52.0492	Route nationale 2	FG	**KY510085**	—	—	—
AF1847	*Anomaloglossus baeobatrachus*	4.1053	-52.0495	Route nationale 2	FG	**KY510086**	—	—	—
AF1848	*Anomaloglossus baeobatrachus*	4.1055	-52.0492	Route nationale 2	FG	**KY510087**	—	—	—
AF1849	*Anomaloglossus baeobatrachus*	4.1055	-52.0495	Route nationale 2	FG	**KY510088**	—	—	—
AF1850	*Anomaloglossus baeobatrachus*	4.1054	-52.0495	Route nationale 2	FG	**KY510089**	—	—	—
AF1851	*Anomaloglossus baeobatrachus*	4.1053	-52.0494	Route nationale 2	FG	**KY510090**	—	—	—
AF1852	*Anomaloglossus baeobatrachus*	4.1053	-52.0494	Route nationale 2	FG	**KY510091**	—	—	—
AF1856	*Anomaloglossus baeobatrachus*	4.1053	-52.0493	Route nationale 2	FG	**KY510092**	—	—	—
AF1889	*Anomaloglossus baeobatrachus*	4.3108	-52.2239	Montagne Petite Tortue	FG	**KY510093**	—	—	—
AF1897	*Anomaloglossus baeobatrachus*	4.3108	-52.2239	Montagne Petite Tortue	FG	**KY510094**	—	—	—
AF1980	*Anomaloglossus stepheni*	2.1134	-56.1742	Sipaliwini	SR	**KY510095**	—	—	—
AF1999	*Anomaloglossus stepheni*	2.1728	-56.0969	Sipaliwini	SR	**KY510096**	—	—	—
AF2019	*Anomaloglossus stepheni*	2.1728	-56.0969	Sipaliwini	SR	**KY510097**	—	—	—
AF2029	*Anomaloglossus leopardus*	2.1697	-56.0808	Sipaliwini	SR	**KY510098**	—	—	—
AF2030	*Anomaloglossus stepheni*	2.1697	-56.0808	Sipaliwini	SR	**KY510099**	—	—	—
AF2031	*Anomaloglossus leopardus*	2.1697	-56.0808	Sipaliwini	SR	**KY510100**	—	—	—
AF2033	*Anomaloglossus leopardus*	2.1697	-56.0808	Sipaliwini	SR	**KY510101**	—	—	—
AF2034	*Anomaloglossus leopardus*	2.1697	-56.0808	Sipaliwini	SR	**KY510102**	—	—	—
AF2035	*Anomaloglossus leopardus*	2.1697	-56.0808	Sipaliwini	SR	**KY510103**	—	—	—
AF2036	*Anomaloglossus leopardus*	2.1697	-56.0808	Sipaliwini	SR	**KY510104**	—	—	—
AF2038	*Anomaloglossus leopardus*	2.1697	-56.0808	Sipaliwini	SR	**KY510105**	—	—	—
AF2039	*Anomaloglossus leopardus*	2.1781	-56.0852	Sipaliwini	SR	**KY510106**	—	—	—
AF2040	*Anomaloglossus leopardus*	2.1781	-56.0852	Sipaliwini	SR	**KY510107**	—	—	—
AF2041	*Anomaloglossus leopardus*	2.1781	-56.0852	Sipaliwini	SR	**KY510108**	**KY549534**	—	**KY549452**

AF2042	*Anomaloglossus leopardus*	2.1781	-56.0852	Sipaliwini	SR	**KY510109**	—	—	—
AF2044	*Anomaloglossus stepheni*	2.1796	-56.0907	Sipaliwini	SR	**KY510110**	—	—	—
AF2045	*Anomaloglossus stepheni*	2.1796	-56.0907	Sipaliwini	SR	**KY510111**	**KY549535**	**KY549495**	**KY549453**
AF2046	*Anomaloglossus stepheni*	2.1796	-56.0907	Sipaliwini	SR	**KY510112**	—	—	—
AF2092	*Anomaloglossus baeobatrachus*	4.0328	-51.9910	Route nationale 2	FG	**KY510113**	**KY549536**	**KY549496**	**KY549454**
AF2093	*Anomaloglossus baeobatrachus*	4.0328	-51.9912	Route nationale 2	FG	**KY510114**	—	—	—
AF2094	*Anomaloglossus baeobatrachus*	4.0327	-51.9910	Route nationale 2	FG	**KY510115**	—	—	—
AF2095	*Anomaloglossus baeobatrachus*	4.0325	-51.9909	Route nationale 2	FG	**KY510116**	—	—	—
AF2096	*Anomaloglossus baeobatrachus*	4.0326	-51.9908	Route nationale 2	FG	**KY510117**	—	—	—
AF2097	*Anomaloglossus baeobatrachus*	4.0329	-51.9907	Route nationale 2	FG	**KY510118**	—	—	—
AF2098	*Anomaloglossus baeobatrachus*	4.0331	-51.9908	Route nationale 2	FG	**KY510119**	—	—	—
AF2099	*Anomaloglossus baeobatrachus*	4.0332	-51.9907	Route nationale 2	FG	**KY510120**	—	—	—
AF2100	*Anomaloglossus baeobatrachus*	4.0346	-51.9915	Route nationale 2	FG	**KY510121**	—	—	—
AF2101	*Anomaloglossus baeobatrachus*	4.0345	-51.9915	Route nationale 2	FG	**KY510122**	—	—	—
AF2102	*Anomaloglossus baeobatrachus*	4.0334	-51.9900	Route nationale 2	FG	**KY510123**	**KY549537**	**KY549497**	**KY549455**
AF2103	*Anomaloglossus baeobatrachus*	4.0334	-51.990	Route nationale 2	FG	**KY510124**	**KY549538**	**KY549498**	**KY549456**
AF2105	*Anomaloglossus baeobatrachus*	4.0332	-51.9907	Route nationale 2	FG	**KY510125**	**KY549539**	**KY549499**	**KY549457**
AF2106	*Anomaloglossus baeobatrachus*	4.0325	-51.9908	Route nationale 2	FG	**KY510126**	**KY549540**	**KY549500**	**KY549458**
AF2217	*Anomaloglossus stepheni*	2.0325	-56.1144	Sipaliwini	SR	**KY510127**	—	—	—
AF2219	*Anomaloglossus stepheni*	2.0325	-56.1144	Sipaliwini	SR	**KY510128**	—	—	—
AF2221	*Anomaloglossus stepheni*	2.0325	-56.1144	Sipaliwini	SR	**KY510129**	—	—	—
AF2314	*Anomaloglossus baeobatrachus*	4.6025	-53.4143	Trinité	FG	**KY510130**	—	—	—
AF2370	*Anomaloglossus surinamensis*	4.7062	-53.9308	Montagne Or	FG	**KY510131**	—	—	—
AF2372	*Anomaloglossus baeobatrachus*	5.1779	-52.7581	CSG	FG	**KY510132**	—	—	—
AF2391	*Anomaloglossus surinamensis*	4.8170	-54.6037	Nassau	SR	**KY510133**	**KY549541**	**KY549501**	**KY549459**
AF2436	*Anomaloglossus stepheni*	4.8041	-54.5555	Nassau	SR	**KY510134**	**KY549542**	**KY549502**	**KY549460**
AF2440	*Anomaloglossus stepheni*	4.8041	-54.5555	Nassau	SR	**KY510135**	—	—	—
AF2456	*Anomaloglossus surinamensis*	4.8172	-54.5909	Nassau	SR	**KY510136**	—	—	—
AF2589	*Anomaloglossus baeobatrachus*	3.5687	-53.9760	Saint-Eugène	FG	—	**KY549543**	**KY549503**	**KY549461**

AF2590	*Anomaloglossus baeobatrachus*	3.5687	-53.9760	Saint-Eugène	FG	**KY510137**	**KY549544**	**KY549504**	**KY549462**
AF2591	*Anomaloglossus baeobatrachus*	3.5687	-53.9760	Saint-Eugène	FG	—	**KY549545**	**KY549505**	**KY549463**
AF2641	*Anomaloglossus baeobatrachus*	3.2181	-52.3966	Monts Atachi Bakka	FG	**KY510138**	—	—	—
AF2673	*Ameerega hahneli*	3.2081	-52.4024	Alikéné	FG	—	—	**KY549506**	**KY549464**
AF2699	*Anomaloglossus baeobatrachus*	2.2357	-54.4492	Alikéné	FG	**KY510139**	—	—	—
AF2726 (APA-973-1-AF2726)	*Anomaloglossus baeobatrachus*	2.23577	-54.4492	Mitaraka	FG	**KY510140**	**KY549546**	**KY549507**	**KY549465**
AF2732 (APA-973-1-AF2732)	*Anomaloglossus* sp. “Mitaraka”	2.2357	-54.4492	Mitaraka	FG	**KY510141**	**KY549547**	**KY549508**	**KY549466**
AF2751 (APA-973-1-AF2751)	*Anomaloglossus* sp. “Mitaraka”	2.2357	-54.4492	Mitaraka	FG	**KY510142**	**KY549548**	**KY549509**	**KY549467**
AF2792 (APA-973-1-AF2792)	*Anomaloglossus surinamensis*	2.2357	-54.4492	Mitaraka	FG	**KY510143**	—	—	—
AF2817 (APA-973-1-AF2817	*Anomaloglossus* sp. “Mitaraka”	2.2357	-54.4492	Mitaraka	FG	**KY510144**	—	—	—
AF2875 (APA-973-1-AF2875)	*Anomaloglossus* sp. “Mitaraka”	2.2357	-54.4492	Mitaraka	FG	**KY510145**	—	—	—
AF2876 (APA-973-1-AF2876)	*Anomaloglossus baeobatrachus*	2.23577	-54.4492	Mitaraka	FG	**KY510146**	—	—	—
AF2877 (APA-973-1-AF2877)	*Anomaloglossus baeobatrachus*	3.3177	-52.1932	Mitaraka	FG	**KY510147**	**KY549549**	**KY549510**	**KY549468**
AF2942	*Anomaloglossus baeobatrachus*	3.3177	-52.1932	Memora G	FG	**KY510148**	—	—	—
AF2963	*Anomaloglossus baeobatrachus*	3.3129	-52.1803	Memora G	FG	**KY510149**	—	—	—
AF3003	*Anomaloglossus baeobatrachus*	3.3129	-52.1803	Mémora D	AP	**KY510150**	—	—	—
AF3017	*Anomaloglossus baeobatrachus*	3.3129	-52.1803	Mémora D	AP	**KY510151**	—	—	—
AF3032	*Anomaloglossus baeobatrachus*	2.6284	-52.5540	Mémora D	AP	**KY510152**	—	—	—
AF3112	*Anomaloglossus baeobatrachus*	2.6284	-52.5540	Mitan G	FG	**KY510153**	—	—	—
AF3116	*Anomaloglossus baeobatrachus*	2.6276	-52.5419	Mitan G	FG	**KY510154**	—	—	—
AF3158	*Anomaloglossus baeobatrachus*	2.6276	-52.519	Mitan D	AP	**KY510155**	—	—	—
AF3159	*Anomaloglossus baeobatrachus*	2.6284	-52.5540	Mitan D	AP	**KY510156**	—	—	—

AF3210	*Anomaloglossus baeobatrachus*	4.7246	-56.7638	Mitan G	FG	**KY510157**	—	—	—
AF3340	*Anomaloglossus surinamensis*	4.6561	-56.7864	Bakhuis	SR	**KY510158**	**KY549550**	**KY549511**	**KY549469**
AF3349	*Anomaloglossus surinamensis*	4.7246	-56.7638	Bakhuis	SR	**KY510159**	**KY549551**	**KY549512**	**KY549470**
AF3412	*Anomaloglossus* sp. “Bakhuis”	4.7246	-56.7638	Bakhuis	SR	**KY510160**	—	—	—
AF3413	*Anomaloglossus* sp. “Bakhuis”	4.7246	-56.7638	Bakhuis	SR	**KY510161**	—	—	—
AF3422	*Anomaloglossus* sp. “Bakhuis”	4.7246	-56.7638	Bakhuis	SR	**KY510162**	—	—	—
AF3424	*Anomaloglossus* sp. “Bakhuis”	4.7246	-56.7638	Bakhuis	SR	**KY510163**	—	—	—
AF3425	*Anomaloglossus* sp. “Bakhuis”	4.7246	-56.7638	Bakhuis	SR	**KY510164**	—	—	—
AF3426	*Anomaloglossus* sp. “Bakhuis”	5.2328	-55.8046	Bakhuis	SR	**KY510165**	**KY549552**	**KY549513**	**KY549471**
AF3450	*Anomaloglossus stepheni*	3.6255	-53.2072	Spari Creek	SR	**KY510166**	—	—	—
AF3541	*Anomaloglossus baeobatrachus*	3.0230	-53.0954	Mont Itoupé	FG	**KY510167**	—	—	—
AF3675	*Anomaloglossus baeobatrachus*	3.0230	-53.0954	Mont Itoupé	FG	**KY510168**	—	—	—
AF3676	*Anomaloglossus baeobatrachus*	3.0230	-53.0954	Mont Itoupé	FG	**KY510169**	—	—	—
AF3677	*Anomaloglossus baeobatrachus*	3.0230	-53.0954	Mont Itoupé	FG	**KY510170**	—	—	—
AF3679	*Anomaloglossus baeobatrachus*	3.0230	-53.0954	Mont Itoupé	FG	**KY510171**	—	—	—
AF3680	*Anomaloglossus baeobatrachus*	3.0230	-53.0954	Mont Itoupé	FG	**KY510172**	—	—	—
AF3685	*Anomaloglossus baeobatrachus*	3.0230	-53.0954	Mont Itoupé	FG	**KY510173**	—	—	—
AF3774	*Anomaloglossus* sp. “Brownsberg”	4.6816	-56.1856	Voltzberg	SR	**KY510174**	—	—	—
AG298	*Anomaloglossus surinamensis*	4.6025	-53.4143	Trinité	FG	JN691047	—	—	—
AG300	*Anomaloglossus baeobatrachus*	4.6025	-53.4143	Trinité	FG	JN690982	—	—	—
AG304	*Anomaloglossus baeobatrachus*	4.6025	-53.4143	Trinité	FG	JN690983	—	—	—
AG305	*Anomaloglossus surinamensis*	4.6025	-53.4143	Trinité	FG	JN691061	—	—	—
AG312	*Anomaloglossus baeobatrachus*	4.6025	-53.4143	Trinité	FG	JN690984	—	—	—
AG362	*Anomaloglossus baeobatrachus*	5.4731	-53.2044	Iracoubo	FG	**KY510175**	—	—	—
AG371	*Anomaloglossus baeobatrachus*	4.4621	-54.3927	Gaa Kaba	FG	**KY510176**	—	—	—
AG372	*Anomaloglossus surinamensis*	4.4621	-54.3927	Gaa Kaba	FG	**KY510177**	—	—	—
AG381	*Anomaloglossus baeobatrachus*	4.8483	-53.8405	Saint-Pierre	FG	**KY510178**	—	—	—
AG392	*Anomaloglossus baeobatrachus*	4.6968	-53.9598	Dekou Dekou	FG	**KY510179**	—	—	—
AG405	*Anomaloglossus surinamensis*	4.8080	-54.1688	Montagnes Sparouine	FG	**KY510180**	—	—	—

AG434	*Anomaloglossus surinamensis*	3.6553	-53.844	Monts Atachi Bakka	FG	**KY510181**	—	—	—
AG441	*Anomaloglossus baeobatrachus*	3.2200	-52.3785	Alikéné	FG	**KY510182**	—	—	—
AG454	*Anomaloglossus baeobatrachus*	3.2200	-52.3785	Alikéné	FG	**KY510183**	—	—	—
AG495	*Anomaloglossus baeobatrachus*	3.7193	-53.4128	Saül Belvedere	FG	**KY510184**	—	—	—
AG499	*Anomaloglossus baeobatrachus*	3.7193	-53.4128	Saül Belvedere	FG	**KY510185**	—	—	—
AJ430675	*Mannophryne collaris*	8.5800	-71.1900	Mérida	VE	AJ430675	—	—	—
AM026	*Anomaloglossus baeobatrachus*	3.6631	-53.9283	Inini Tolenga	FG	**KY510186**	—	—	—
AY263237	*Anomaloglossus stepheni*	-2.9800	-59.9300	Reserva Duke, Manaus	AM	AY263237	—	—	—
BOAM011	*Anomaloglossus baeobatrachus*	2.3709	-53.7728	Borne4	FG	**KY510187**	—	—	—
BOAM018	*Anomaloglossus baeobatrachus*	2.3709	-53.7728	Borne4	FG	**KY510188**	—	—	—
BOAM019	*Anomaloglossus baeobatrachus*	2.3709	-53.7728	Borne4	FG	**KY510189**	—	—	—
BOAM023	*Anomaloglossus baeobatrachus*	2.3709	-53.7728	Borne4	FG	**KY510190**	—	—	—
BOAM043	*Anomaloglossus baeobatrachus*	2.3709	-53.7728	Borne4	FG	**KY510191**	—	—	—
BOAM044	*Anomaloglossus baeobatrachus*	2.3709	-53.7728	Borne4	FG	**KY510192**	—	—	—
BOAM055	*Anomaloglossus baeobatrachus*	2.3709	-53.7728	Borne4	FG	**KY510193**	—	—	—
BPN0836	*Anomaloglossus stepheni*	3.7833	-56.1500	Tafelberg	SR	JN691112	—	—	—
BPN0837	*Anomaloglossus surinamensis*	3.7833	-56.1500	Tafelberg	SR	JN691099	—	—	—
BPN0849	*Anomaloglossus* sp. “Brownsberg”	4.9365	-55.1948	Brownsberg	SR	JN691037	—	—	—
BPN0850	*Anomaloglossus* sp. “Brownsberg”	4.9365	-55.1948	Brownsberg	SR	JN691039	JN691716	KY549514	KY549472
BPN0851	*Anomaloglossus* sp. “Brownsberg”	4.9365	-55.1948	Brownsberg	SR	JN691038	—	—	—
BPN0852	*Anomaloglossus* sp. “Brownsberg”	4.9365	-55.1948	Brownsberg	SR	JN691040	—	—	—
BPN0853	*Anomaloglossus* sp. “Brownsberg”	4.9365	-55.1948	Brownsberg	SR	JN691041	—	—	—
BPN1063	*Anomaloglossus stepheni*	4.2666	-54.7333	Lely Mountain	SR	JN691111	—	—	—
BPN1299	*Anomaloglossus megacephalus*	5.7423	-60.2991	Mount Thomasing	GY	JN691123	—	—	—
BPN1304	*Anomaloglossus megacephalus*	5.7423	-60.2991	Mount Thomasing	GY	JN691124	—	—	—
BPN1305	*Anomaloglossus megacephalus*	5.8209	-60.1714	Mount Thomasing	GY	JN691125	—	—	—
BPN1369	*Anomaloglossus* sp. “north FG”	4.5161	-52.1005	Montagne de Kaw	FG	JN691110	—	—	—
BPN1480	*Anomaloglossus baeobatrachus*	4.5161	-52.1005	Montagne de Kaw	FG	JN690970	—	—	—
BPN1629	*Anomaloglossus baeobatrachus*	3.6255	-53.2072	Saül	FG	JN690968	—	—	—

BPN1699	*Anomaloglossus baeobatrachus*	3.6255	-53.2072	Saül	FG	JN690969	—	—	—
BPN2954	*Anomaloglossus* sp. “Mitaraka”	2.9770	-55.4080	Kasikasima	SR	**KY510194**	—	—	—
BPN2967	*Anomaloglossus leopardus*	2.4788	-55.6316	Kasikasima	SR	**KY510195**	—	—	—
BPN2974	*Anomaloglossus* sp. “Mitaraka”	2.9860	-55.3869	Kasikasima	SR	**KY510196**	—	—	—
CAAM02	*Anomaloglossus baeobatrachus*	2.3491	-53.2159	Montagne Cacao	FG	**KY510197**	—	—	—
CAAM03	*Anomaloglossus baeobatrachus*	2.3491	-53.2159	Montagne Cacao	FG	**KY510198**	—	—	—
CAAM35	*Anomaloglossus baeobatrachus*	2.3491	-53.2159	Montagne Cacao	FG	**KY510199**	—	—	—
CAAM36	*Anomaloglossus baeobatrachus*	2.3491	-53.2159	Montagne Cacao	FG	**KY510200**	—	—	—
CLBA-Voucher	*Anomaloglossus rufulus*	5.3000	-62.1667	Churi Tepui	VE	KJ940456	—	—	—
CM059	*Anomaloglossus baeobatrachus*	3.3005	-52.9493	Monts Bakra	FG	JN690967	—	—	—
CM060	*Anomaloglossus baeobatrachus*	3.3005	-52.9493	Monts Bakra	FG	JN690971	—	—	—
CM068	*Anomaloglossus surinamensis*	3.3005	-52.9493	Monts Bakra	FG	EU201073	—	—	—
CM069	*Anomaloglossus surinamensis*	3.3005	-52.9493	Monts Bakra	FG	JN691065	—	—	—
CM110	*Anomaloglossus* sp. “north FG”	4.4166	-52.3000	Tibourou	FG	JN691106	—	—	—
CM113	*Anomaloglossus* sp. “north FG”	4.4166	-52.3000	Tibourou	FG	EU201079	—	—	—
CM148	*Anomaloglossus baeobatrachus*	2.3333	-54.6000	Trijonction	FG	EU201072	—	—	—
CM182	*Anomaloglossus* sp. “Mitaraka”	2.3333	-54.6000	Trijonction	FG	EU201071	—	—	—
CM220	*Anomaloglossus baeobatrachus*	3.6255	-53.2072	Saül	FG	EU201070	—	—	—
CM230	*Anomaloglossus degranvillei*	3.6255	-53.2072	Saül	FG	EU201080	—	—	—
CM236	*Anomaloglossus baeobatrachus*	4.8333	-53.2500	Piste Saint-Elie	FG	JN690973	—	—	—
CM238	*Anomaloglossus baeobatrachus*	5.283611	-53.0538	Piste Saint-Elie	FG	JN690993	—	—	—
CM266	*Anomaloglossus baeobatrachus*	4.0500	-52.0166	DZ5	FG	JN690974	—	—	—
CM295	*Anomaloglossus baeobatrachus*	4.8936	-52.8047	Camp Canope	FG	JN690996	—	—	—
CM305	*Anomaloglossus baeobatrachus*	3.0521	-52.7050	Toponowini	FG	JN691021	—	—	—
CM329	*Anomaloglossus* sp. “north FG”	4.3000	-52.3666	Montagne Petite Tortue	FG	JN691102	—	—	—
CM346	*Anomaloglossus baeobatrachus*	4.5161	-52.1005	Montagne de Kaw	FG	JN690994	—	—	—
CM355	*Anomaloglossus surinamensis*	4.7774	-53.9475	Lucifer	FG	EU201075	—	—	—
CM357	*Anomaloglossus surinamensis*	4.7774	-53.9475	Lucifer	FG	JN691064	—	—	—
CM382	*Anomaloglossus baeobatrachus*	4.3000	-52.1166	Regina	FG	JN691002	—	—	—

CM414	*Anomaloglossus baeobatrachus*	4.7774	-53.9475	Lucifer	FG	JN691023	—	—	—
CM424	*Anomaloglossus baeobatrachus*	4.5666	-52.4666	Molokoi	FG	JN690990	—	—	—
CPI10198	*Anomaloglossus praderioi*	5.2332	-60.7425	Mazaruni-Potoro, Mt. Roraima	GY	DQ502255	—	—	—
CPI10208	*Anomaloglossus praderioi*	5.2332	-60.7425	Mazaruni-Potoro, Mt. Roraima	GY	DQ502256	—	—	—
CPI10209	*Anomaloglossus kaiei*	5.2332	-60.7425	Mazaruni-Potoro, Mt. Roraima	GY	DQ502257	—	—	—
CPI10216	*Anomaloglossus roraima*	5.2332	-60.7425	Mazaruni-Potoro, Mt. Roraima	GY	DQ502258	—	—	—
CPI10217	*Anomaloglossus roraima*	5.2332	-60.7425	Mazaruni-Potoro, Mt. Roraima	GY	DQ502259	—	—	—
CVULA7399	*Aromobates meridiensis*	11.1200	-69.7000	Altos de San Luis, near La Asu	VE	—	—	JX036006	
CVULA8321	*Aromobates saltuensis*	7.5787	-72.1790	Rio Negro, Parque Nacional El Tama	VE	JX035996	—	—	—
DQ502019	*Anomaloglossus kaiei*	5.8169	-60.0747	Mereme Mountains	GY	DQ502019	—	—	—
DQ502020	*Anomaloglossus kaiei*	5.8169	-60.0747	Mereme Mountains	GY	DQ502020	—	—	—
FL22	*Anomaloglossus baeobatrachus*	0.9793	-51.6148	Porto Grande	AP	**KY510201**	—	—	—
IRSNB13741	*Anomaloglossus beebei*	5.2686	-59.7686	Kaieteur	GY	JQ742107	—	—	—
IRSNB13752	*Anomaloglossus beebei*	5.2686	-59.7686	Kaieteur	GY	JQ742108	—	—	—
IRSNB14410	*Anomaloglossus praderioi*	5.2124	-60.5758	Maringma Tepui	GY	JQ742106	—	—	—
IRSNB14454	*Anomaloglossus kaiei*	5.2686	-59.7686	Kaieteur	GY	JQ742110	—	—	—
IRSNB15849	*Anomaloglossus* sp. “north FG”	4.5161	-52.1005	Montagne de Kaw	FG	JQ742125	—	—	—
IRSNB15851	*Anomaloglossus roraima*	5.2291	-60.7120	Wei Assipu Tepui	GY	JQ742113	—	—	—
IRSNB15864	*Anomaloglossus roraima*	5.2124	-60.5758	Maringma Tepui	GY	JQ742111	—	—	—
IRSNB15865	*Anomaloglossus roraima*	5.2291	-60.7120	Wei Assipu Tepui	GY	JQ742112	—	—	—
IRSNB1986	*Anomaloglossus megacephalus*	5.2124	-60.5758	Maringma Tepui	GY	JQ742105	—	—	—
JPL301	*Anomaloglossus baeobatrachus*	4.8502	-52.3480	Matoury	FG	**KY510203**	—	—	—
LC30	*Anomaloglossus baeobatrachus*	2.3167	-51.6285	Lourenço	AP	**KY510204**	—	—	—
LPT34	*Anomaloglossus baeobatrachus*	-0.6008	-52.3888	Laranjal do Jari	AP	**KY510205**	—	—	—
MB120	*Anomaloglossus surinamensis*	3.6255	-53.2072	Saül	FG	JN691077	—	—	—
MB125	*Anomaloglossus surinamensis*	3.6255	-53.2072	Saül	FG	EU201074	—	—	—
MB134	*Anomaloglossus baeobatrachus*	4.2000	-52.1666	Mataroni	FG	JN690972	—	—	—
MB217	*Anomaloglossus baeobatrachus*	4.5161	-52.1005	Montagne de Kaw	FG	JN690997	—	—	—

MHUA5357	*Rheobates pseudopalmatus*	6.97800	-75.1111	Antioquia, Anori, Reserva Natural El Arrierito	CO	KJ130728	—	—	—
MJH3928	*Anomaloglossus stepheni*	-2.9661	-59.9312	Reserva Florestal Adolfo Ducke	AM	DQ502107	—	—	—
MJH3950	*Anomaloglossus stepheni*	-2.9661	-59.9312	Reserva Florestal Adolfo Ducke	AM	DQ502108	—	—	—
MNHN1995-9454	*Anomaloglossus baeobatrachus*	3.9904	-52.5766	Arataye	FG	AY263236	—	—	—
MNHN2000-654	*Anomaloglossus baeobatrachus*	3.6255	-53.2072	Saül	FG	AY263231	—	—	—
MNHN2000-655	*Anomaloglossus surinamensis*	3.6255	-53.2072	Saül	FG	AY263260	—	—	—
MNRJ38049	*Anomaloglossus tamacuarensis*	1.2092	-64.7483	Pico Tamacuari	VE	**KY510206**	—	—	—
MPEG23103	*Anomaloglossus stepheni*	-1.71401	-57.2133	Faro	PA	**KY510207**	—	—	—
MPEG23108	*Anomaloglossus stepheni*	-1.71401	-57.2133	Faro	PA	**KY510208**	—	—	—
MPEG23109	*Anomaloglossus stepheni*	-1.71401	-57.2133	Faro	PA	**KY510209**	—	—	—
MPEG23111	*Anomaloglossus stepheni*	-1.71401	-57.2133	Faro	PA	**KY510210**	—	—	—
MPEG23113	*Anomaloglossus stepheni*	-1.71401	-57.2133	Faro	PA	**KY510211**	—	—	—
MPEG29853	*Anomaloglossus stepheni*	-0.9627	-55.5223	Trombetas	PA	**KY510212**	—	—	—
MPEG30205	*Anomaloglossus baeobatrachus*	1.2854	-58.6959	Serra do Acari	PA	**KY510213**	—	—	—
MPEG30212	*Anomaloglossus baeobatrachus*	1.2854	-58.6959	Serra do Acari	PA	**KY510214**	—	—	—
MPEG30218	*Anomaloglossus baeobatrachus*	1.2854	-58.6959	Serra do Acari	PA	**KY510215**	—	—	—
MPEG30219	*Anomaloglossus baeobatrachus*	1.2854	-58.6959	Serra do Acari	PA	**KY510216**	—	—	—
MPEG30224	*Anomaloglossus baeobatrachus*	1.2854	-58.6959	Serra do Acari	PA	**KY510217**	—	—	—
MPEG30242	*Anomaloglossus baeobatrachus*	1.2854	-58.6959	Serra do Acari	PA	**KY510218**	—	—	—
MPEG30247	*Anomaloglossus baeobatrachus*	1.2854	-58.6959	Serra do Acari	PA	**KY510219**	—	—	—
MPEG30248	*Anomaloglossus baeobatrachus*	1.2854	-58.6959	Serra do Acari	PA	**KY510220**	—	—	—
MPEG30253	*Anomaloglossus baeobatrachus*	1.2854	-58.6959	Serra do Acari	PA	**KY510221**	—	—	—
MPEG30541	*Anomaloglossus baeobatrachus*	-0.9439	-53.2363	Parú	PA	**KY510222**	—	—	—
MPEG30542	*Anomaloglossus baeobatrachus*	-0.9439	-53.2363	Parú	PA	**KY510223**	—	—	—
MPEG30543	*Anomaloglossus baeobatrachus*	-0.9439	-53.2363	Parú	PA	**KY510224**	—	—	—
MPEG30545	*Anomaloglossus baeobatrachus*	-0.9439	-53.2363	Parú	PA	**KY510225**	—	—	—
MSH10334	*Anomaloglossus stepheni*	-2.5344	-60.8366	E.E. Anavilhanas	AM	**KY510226**	—	—	—
MSH10336	*Anomaloglossus stepheni*	-2.5344	-60.8366	E.E. Anavilhanas	AM	**KY510227**	—	—	—

MTR06278	*Anomaloglossus baeobatrachus*	-0.0241	-51.8972	Igarape Camaipi	AP	JN691015	—	—	—
MTR10269	*Anomaloglossus stepheni*	-3.0088	-60.3969	Igarape Araras	AM	JN691117	—	—	—
MTR10270	*Anomaloglossus stepheni*	-3.0088	-60.3969	Igarape Araras	AM	JN691118	—	—	—
MTR13715	*Anomaloglossus baeobatrachus*	0.9180	-52.0027	Serra do Navio	AP	JN691006	—	—	—
MTR13716	*Anomaloglossus baeobatrachus*	0.9180	-52.0027	Serra do Navio	AP	JN691007	—	—	—
MTR13717	*Anomaloglossus baeobatrachus*	0.9180	-52.0027	Serra do Navio	AP	JN691008	—	—	—
MTR13718	*Anomaloglossus baeobatrachus*	0.9180	-52.0027	Serra do Navio	AP	**KY510228**	—	—	—
MTR13721	*Anomaloglossus baeobatrachus*	0.9180	-52.0027	Serra do Navio	AP	JN691009	JN691647	**KY549516**	**KY549474**
MTR13723	*Anomaloglossus baeobatrachus*	0.9180	-52.0027	Serra do Navio	AP	JN691010	JN691648	**KY549517**	**KY549475**
MTR13724	*Anomaloglossus baeobatrachus*	0.9180	-52.0027	Serra do Navio	AP	JN691011	JN691649	**KY549518**	**KY549476**
MTR13805	*Anomaloglossus baeobatrachus*	0.9180	-52.0027	Serra do Navio	AP	JN691012	—	—	—
MTR13833	*Anomaloglossus baeobatrachus*	0.9180	-52.0027	Serra do Navio	AP	JN691014	—	—	—
MTR13856	*Anomaloglossus baeobatrachus*	2.3236	-51.6452	Lourenço	AP	JN691024	—	—	—
MTR13857	*Anomaloglossus baeobatrachus*	2.3236	-51.6452	Lourenço	AP	JN691026	JN691652	**KY549519**	**KY549477**
MTR13861	*Anomaloglossus baeobatrachus*	2.3236	-51.6452	Lourenço	AP	JN691025	JN691653	**KY549520**	**KY549478**
MTR13862	*Anomaloglossus baeobatrachus*	2.3236	-51.6452	Lourenço	AP	JN691027	JN691657	—	—
MTR13863	*Anomaloglossus baeobatrachus*	2.3236	-51.6452	Lourenço	AP	JN691028	JN691659	**KY549521**	**KY549479**
MTR13877	*Anomaloglossus baeobatrachus*	2.3236	-51.6452	Lourenço	AP	JN691029	JN691661	**KY549522**	**KY549480**
MTR13879	*Anomaloglossus baeobatrachus*	2.3236	-51.6452	Lourenço	AP	JN691030	—	—	—
MTR13887	*Anomaloglossus baeobatrachus*	2.3236	-51.6452	Lourenço	AP	JN691016	JN691663	—	—
MTR13929	*Anomaloglossus baeobatrachus*	2.3236	-51.6452	Lourenço	AP	JN691031	JN691658	**KY549523**	**KY549481**
MTR13959	*Allobates femoralis*	-0.6008	-52.3888	Laranjal do Jari	AP	—	JN691621	—	—
MTR16435	*Allobates alagoanus*	-14.3655	-39.1086	Itacaré	BA	—	**KY549554**	—	**KY549482**
MTR23223	*Anomaloglossus apiau*	2.3803	-61.3576	Serra da Maroquinha	RR	**KY510229**	**KY549555**	**KY549524**	**KY549483**
MTR23338	*Anomaloglossus apiau*	2.3803	-61.3576	Serra da Maroquinha	RR	**KY510230**	—	—	—
MTR24090	*Anomaloglossus baeobatrachus*	3.8597	-51.760	Oiapoque	AP	**KY510231**	—	—	—
MTR24144	*Anomaloglossus baeobatrachus*	3.8794	-51.7709	Oiapoque	AP	KR811196	—	—	—
MTR24186	*Anomaloglossus baeobatrachus*	3.7960	-51.8629	Oiapoque	AP	**KY510232**	—	—	—
MTR24210	*Anomaloglossus baeobatrachus*	2.3526	-51.6152	Lourenço	AP	KR811195	—	—	—

MTR24258	*Anomaloglossus baeobatrachus*	2.3215	-51.6108	Lourenço	AP	**KY510233**	—	—	—
MUJ3726	*Aromobates saltuensis*	6.9900	-72.1000	Boyaca, Cubara	CO	—	—	—	DQ503406
NZCS073	*Anomaloglossus stepheni*	5.1243	-55.2492	Rosebel	SR	**KY510234**	—	—	—
NZCS077	*Anomaloglossus stepheni*	5.3833	-55.5997	Coesewijne savanne	SR	**KY510235**	—	—	—
OPC26	*Anomaloglossus baeobatrachus*	3.8355	-51.8333	Oiapoque	AP	**KY510236**	**KY549556**	**KY549525**	**KY549484**
PG091	*Anomaloglossus baeobatrachus*	2.3887	-53.0144	Mont Saint Marcel	FG	JN691004	—	—	—
PG092	*Anomaloglossus baeobatrachus*	2.3887	-53.0144	Mont Saint Marcel	FG	JN690966	—	—	—
PG106	*Anomaloglossus baeobatrachus*	2.3887	-53.0144	Mont Saint Marcel	FG	JN690964	—	—	—
PG107	*Anomaloglossus baeobatrachus*	2.3887	-53.0144	Mont Saint Marcel	FG	JN690965	—	—	—
PG124	*Anomaloglossus baeobatrachus*	3.0521	-52.7050	Toponowini	FG	JN691001	—	—	—
PG127	*Anomaloglossus baeobatrachus*	3.0521	-52.7050	Toponowini	FG	JN691017	—	—	—
PG128	*Anomaloglossus baeobatrachus*	3.0521	-52.7050	Toponowini	FG	JN691018	—	—	—
PG163	*Anomaloglossus* sp. “north FG”	3.8044	-52.2880	Armontabo	FG	JN691104	—	—	—
PG164	*Anomaloglossus* sp. “north FG”	3.8044	-52.2880	Armontabo	FG	JN691105	—	—	—
PG239	*Anomaloglossus baeobatrachus*	2.5134	-53.8211	Haute Wanapi	FG	JN691022	—	—	—
PG246	*Anomaloglossus baeobatrachus*	2.5134	-53.8211	Haute Wanapi	FG	JN691003	—	—	—
PG302	*Anomaloglossus* sp. “Mitaraka”	2.6067	-54.0257	Haut Marwini	FG	JN691035	—	—	—
PG303	*Anomaloglossus baeobatrachus*	2.6067	-54.0257	Haut Marwini	FG	EU201072	—	—	—
PG337A	*Anomaloglossus surinamensis*	3.3005	-52.9493	Monts Bakra	FG	JN691071	—	—	—
PG337B	*Anomaloglossus surinamensis*	3.3005	-52.9493	Monts Bakra	FG	JN691066	—	—	—
PG337C	*Anomaloglossus surinamensis*	3.3005	-52.9493	Monts Bakra	FG	JN691074	—	—	—
PG337D	*Anomaloglossus surinamensis*	3.3005	-52.9493	Monts Bakra	FG	JN691073	—	—	—
PG337E	*Anomaloglossus surinamensis*	3.3005	-52.9493	Monts Bakra	FG	JN691067	—	—	—
PG337F	*Anomaloglossus surinamensis*	3.3005	-52.9493	Monts Bakra	FG	JN691068	—	—	—
PG337G	*Anomaloglossus surinamensis*	3.3005	-52.9493	Monts Bakra	FG	JN691069	—	—	—
PG337H	*Anomaloglossus surinamensis*	3.3005	-52.9493	Monts Bakra	FG	JN691070	—	—	—
PG337I	*Anomaloglossus surinamensis*	3.3005	-52.9493	Monts Bakra	FG	JN691072	—	—	—
PG393	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691095	—	—	—
PG394	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691093	—	—	—

PG395	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691094	—	—	—
PG407	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691084	—	—	—
PG457	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691085	—	—	—
PG458	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691091	—	—	—
PG459	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691086	—	—	—
PG460	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691087	—	—	—
PG461	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691092	—	—	—
PG462	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691088	—	—	—
PG463	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691090	—	—	—
PG464	*Anomaloglossus surinamensis*	3.9347	-54.2047	Mont Kotika	FG	JN691089	—	—	—
PG465	*Anomaloglossus baeobatrachus*	3.9347	-54.2047	Mont Kotika	FG	JN691019	—	—	—
PG466	*Anomaloglossus baeobatrachus*	3.9347	-54.2047	Mont Kotika	FG	JN691020	—	—	—
PG517	*Anomaloglossus surinamensis*	3.3005	-52.9493	Pic Coudreau	FG	**KY510237**	—	—	—
PG518	*Anomaloglossus surinamensis*	3.3005	-52.9493	Pic Coudreau	FG	**KY510238**	—	—	—
PG531	*Anomaloglossus baeobatrachus*	2.5134	-53.8211	Haute Wanapi	FG	**KY510239**	—	—	—
PG532	*Anomaloglossus baeobatrachus*	2.5134	-53.8211	Haute Wanapi	FG	**KY510240**	—	—	—
PG533	*Anomaloglossus baeobatrachus*	2.5134	-53.8211	Haute Wanapi	FG	**KY510241**	—	—	—
PG542	*Anomaloglossus baeobatrachus*	2.5134	-53.8211	Haute Wanapi	FG	**KY510242**	—	—	—
PG572	*Anomaloglossus baeobatrachus*	2.2911	-54.5342	Mitaraka	FG	JN691013	—	—	—
PG573	*Anomaloglossus* sp. “Mitaraka”	2.2911	-54.5342	Mitaraka	FG	JN691032	—	—	—
PG590	*Anomaloglossus* sp. “Mitaraka”	2.2911	-54.5342	Mitaraka	FG	JN691034	—	—	—
PG592	*Anomaloglossus* sp. “Mitaraka”	2.2911	-54.5342	Mitaraka	FG	EU201071	—	—	—
PG593	*Anomaloglossus* sp. “Mitaraka”	2.2911	-54.5342	Mitaraka	FG	JN691033	—	—	—
PG601	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510243**	**KY549557**	**KY549526**	**KY549485**
PG602	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510244**	—	—	—
PG603	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510245**	**KY549558**	**KY549527**	**KY549486**
PG604	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510246**	—	—	—
PG605	*Anomaloglossus surinamensis*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510247**	—	—	—
PG606	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510248**	—	—	—

PG607	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510249**	—	—	—
PG608	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510250**	—	—	—
PG609	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510251**	—	—	—
PG610	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510252**	—	—	—
PG611	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510253**	—	—	—
PG612	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510254**	—	—	—
PG620	*Anomaloglossus surinamensis*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510255**	—	—	—
PG621	*Anomaloglossus surinamensis*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510256**	—	—	—
PG622	*Anomaloglossus surinamensis*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510257**	—	—	—
PG624	*Anomaloglossus surinamensis*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510258**	—	—	—
PG626	*Anomaloglossus surinamensis*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510259**	—	—	—
PG627	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510260**	—	—	—
PG628	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510261**	—	—	—
PG629	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510262**	—	—	—
PG631	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510263**	—	—	—
PG632	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510264**	—	—	—
PG633	*Anomaloglossus degranvillei*	3.5454	-53.9068	Monts Atachi Bakka	FG	**KY510265**	—	—	—
PG659	*Anomaloglossus* sp. “Itoupé”	3.0250	-53.0800	Itoupé	FG	**KY510266**	—	—	—
PG660	*Anomaloglossus* sp. “Itoupé”	3.0250	-53.0800	Itoupé	FG	**KY510267**	—	—	—
PG720	*Anomaloglossus baeobatrachus*	3.8061	-51.8933	Saut Maripa	FG	KR811194	—	—	—
PG790	*Anomaloglossus baeobatrachus*	4.1959	-52.1490	Savane Virginie	FG	KR811193	—	—	—
PG791	*Anomaloglossus baeobatrachus*	4.1959	-52.1490	Savane Virginie	FG	**KY510268**	—	—	—
PG792	*Anomaloglossus surinamensis*	5.4280	-53.8872	Sainte-Anne	FG	**KY510269**	—	—	—
PK-437-1	*Anomaloglossus baeobatrachus*	3.7500	-53.0333	Pic Matecho	FG	DQ501980	—	—	—
PK-437-2	*Anomaloglossus baeobatrachus*	3.7500	-53.0333	Pic Matecho	FG	DQ501981	—	—	—
PK-437-3	*Anomaloglossus baeobatrachus*	3.7500	-53.0333	Pic Matecho	FG	DQ501982	—	—	—
PK-437-4	*Anomaloglossus baeobatrachus*	3.7500	-53.0333	Pic Matecho	FG	DQ501983	—	—	—
PK01159	*Anomaloglossus kaiei*	5.2686	-59.7686	Kaieteur	GY	JN691119	—	—	—
PK01303	*Anomaloglossus kaiei*	5.2681	-59.7686	Kaieteur	GY	JN691121	—	—	—

PK0933	*Anomaloglossus beebei*	5.2686	-59.7686	Kaieteur	GY	JN691122	JN691804	—	—
PK1159	*Anomaloglossus kaiei*	5.2681	-59.7686	Kaieteur	GY	—	JN691805	—	—
PK12087	*Anomaloglossus kaiei*	5.2686	-59.7686	Kaieteur	GY	JN691120	—	—	—
PK1304	*Anomaloglossus megacephalus*	5.7423	-60.2991	Mount Thomasing	GY	—	JN691802	—	—
PK1367	*Anomaloglossus kaiei*	5.1329	-59.4166	Kinky Backdam, Kaieteur	GY	**KY510270**	—	**KY549528**	**KY549487**
PK1881	*Anomaloglossus megacephalus*	5.2102	-60.5663	Maringma Tepui	GY	**KY510271**	—	**KY549529**	**KY549488**
PK1991	*Anomaloglossus praderioi*	5.2044	-60.5775	Maringma Tepui	GY	**KY510272**	—	**KY549530**	**KY549489**
PK2046	*Anomaloglossus roraima*	5.2163	-60.5847	Maringma Tepui	GY	**KY510273**	—	—	—
QCAZ16484	*Allobates femoralis*	-1.0700	-75.6900	Parque Nacional Yasuni-Estacion PUCE	EC	—	—	HQ290831	
QCAZ19240	*Ameerega hahneli*	-1.0700	-75.6900	Parque Nacional Yasuni-Estacion PUCE	EC	—	HQ290935	—	—
RB01	*Anomaloglossus surinamensis*	3.7500	-53.0333	Pic Matecho	FG	JN691075	—	—	—
RB02	*Anomaloglossus surinamensis*	3.7500	-53.0333	Pic Matecho	FG	JN691076	—	—	—
RB17	*Anomaloglossus surinamensis*	4.8500	-53.0666	Saint-Eugène	FG	EU201076	—	—	—
RB23	*Anomaloglossus baeobatrachus*	4.8500	-53.0666	Saint-Eugène	FG	JN691000	—	—	—
RB34	*Anomaloglossus surinamensis*	4.8500	-53.0666	Saint-Eugène	FG	JN691063	—	—	—
RB36	*Anomaloglossus baeobatrachus*	4.8500	-53.0666	Saint-Eugène	FG	JN690998	—	—	—
RB37	*Anomaloglossus baeobatrachus*	4.8500	-53.0666	Saint-Eugène	FG	JN690999	—	—	—
RBE49-3	*Anomaloglossus baeobatrachus*	3.6255	-53.2072	Saül	FG	EU201070	—	—	—
RBE49-6	*Anomaloglossus surinamensis*	3.6255	-53.2072	Saül	FG	JN691082	—	—	—
RF33	*Anomaloglossus stepheni*	-2.7044	-58.2494	Silves	AM	**KY510274**	—	—	—
ROM39631	*Anomaloglossus beebei*	5.3822	-59.9768	Mount Ayanganna	GY	DQ502127	—	—	—
ROM39632	*Anomaloglossus beebei*	5.3822	-59.9768	Mount Ayanganna	GY	DQ502130	—	—	—
ROM39635	*Anomaloglossus beebei*	5.3995	-59.9505	Mount Ayanganna	GY	JQ742109	—	—	—
ROM39637	*Anomaloglossus tepuyensis*	5.3822	-59.9768	Mount Ayanganna	GY	DQ502128	—	—	—
ROM39639	*Anomaloglossus* sp. “Ayangana”	5.4000	-59.9500	Mount Ayanganna	GY	DQ502129	—	—	—
ROM43320	*Anomaloglossus* sp. B	5.0829	-59.8487	Wokomung Massif	GY	JQ742114	—	—	—
ROM43323	*Anomaloglossus* sp. B	5.0829	-59.8487	Wokomung Massif	GY	JQ742115	—	—	—
ROM43327	*Anomaloglossus kaiei*	5.0829	-59.8487	Wokomung Massif	GY	JQ742116	—	—	—

ROM43333	*Anomaloglossus kaiei*	5.0829	-59.8487	Wokomung Massif	GY	JQ742117	—	—	—
ROM43892	*Anomaloglossus* sp. A	5.0829	-59.8487	Wokomung Massif	GY	JQ742119	—	—	—
ROM43902	*Anomaloglossus* sp. A	5.0829	-59.8487	Wokomung Massif	GY	JQ742118	—	—	—
ROM44102	*Anomaloglossus kaiei*	6.3532	-60.3557	Meamu River	GY	JQ742123	—	—	—
ROM44104	*Anomaloglossus kaiei*	6.3532	-60.3557	Meamu River	GY	JQ742124	—	—	—
ROM44110	*Anomaloglossus* sp. C	6.1357	-60.3838	Seroun River	GY	JQ742122	—	—	—
ROM44112	*Anomaloglossus* sp. C	5.8169	-60.0747	Mereme Mountains	GY	JQ742120	—	—	—
ROM44113	*Anomaloglossus* sp. C	6.3532	-60.3557	Meamu River	GY	JQ742121	—	—	—
SMS213	*Anomaloglossus stepheni*	-4.9136	-61.1091	Campo Catuquira	AM	**KY510275**	—	—	—
SMS953	*Anomaloglossus apiau*	2.4334	-61.4138	Apiau	RR	**KY510276**	—	—	—
ST282	*Anomaloglossus baeobatrachus*	0.9091	-53.2284	Tumucumaque	AP	**KY510277**	—	—	—
ST294	*Anomaloglossus baeobatrachus*	0.9091	-53.2284	Tumucumaque	AP	**KY510278**	—	—	—
T-2532	*Anomaloglossus baeobatrachus*	3.7500	-53.0333	Pic Matecho	FG	EU201070	—	—	—
T-2533	*Anomaloglossus baeobatrachus*	3.7500	-53.0333	Pic Matecho	FG	EU201070	—	—	—
T-2535	*Anomaloglossus surinamensis*	3.7500	-53.0333	Pic Matecho	FG	JN691079	—	—	—
T-2536	*Anomaloglossus surinamensis*	3.7500	-53.0333	Pic Matecho	FG	JN691078	—	—	—
T-2563	*Anomaloglossus surinamensis*	3.7500	-53.0333	Pic Matecho	FG	JN691080	—	—	—
T-2564	*Anomaloglossus surinamensis*	3.7500	-53.0333	Pic Matecho	FG	JN691081	—	—	—
T-2565	*Anomaloglossus baeobatrachus*	3.7500	-53.0333	Pic Matecho	FG	EU201070	—	—	—
T-2566	*Anomaloglossus baeobatrachus*	3.7500	-53.0333	Pic Matecho	FG	EU201070	—	—	—
T-3023	*Anomaloglossus* sp. “Mitaraka”	2.2666	-54.5166	Mitaraka	FG	EU201071	—	—	—
T-3024	*Anomaloglossus* sp. “Mitaraka”	2.2666	-54.5166	Mitaraka	FG	EU201071	—	—	—
T-3025	*Anomaloglossus surinamensis*	2.2666	-54.5166	Mitaraka	FG	EU201077	—	—	—
T-3026	*Anomaloglossus surinamensis*	2.2666	-54.5166	Mitaraka	FG	JN691100	—	—	—
T-3029	*Anomaloglossus surinamensis*	2.2666	-54.5166	Mitaraka	FG	JN691101	—	—	—
T-3030	*Anomaloglossus baeobatrachus*	2.2666	-54.5166	Mitaraka	FG	JN691005	—	—	—
T-3031	*Anomaloglossus* sp. “Mitaraka”	2.2666	-54.5166	Mitaraka	FG	JN691036	—	—	—
T-3033	*Anomaloglossus* sp. “Mitaraka”	2.2666	-54.5166	Mitaraka	FG	EU201071	—	—	—
T-3040	*Anomaloglossus baeobatrachus*	2.2666	-54.5166	Mitaraka	FG	EU201072	—	—	—

TJC1090	*Anomaloglossus kaiei*	5.10671	-59.6355	Potaro-Siparuni	GY	**KY510279**	—	—	—
TJC1103	*Anomaloglossus beebei*	5.1797	-59.4855	Kaieteur	GY	**KY510280**	—	**KY549531**	**KY549490**
TJC1140	*Anomaloglossus kaiei*	5.2738	-59.5185	Muri Muri	GY	**KY510281**	—	—	—
TNHCFS5507	*Mannophryne collaris*	8.5800	-71.1900	Merida, El Estanquillo	VE	—	HQ290941	—	—
TNHCFS5523	*Mannophryne collaris*	8.5800	-71.1900	Mérida	VE	—	—	FS5523	FS5523
TNHCFS5541	*Aromobates saltuensis*	8.0900	-72.2400	Tachira, San Felix, San Juan de Colon	VE	—	HQ290908	—	—
TNHCFS5627	*Anomaloglossus verbeeksnyderorum*	5.3855	-67.6145	Amazonas: Tobogán de la Selva	VE	**KY510202**	**KY549553**	**KY549515**	**KY549473**
TNHCFS5631	*Anomaloglossus verbeeksnyderorum*	5.6513	-67.4988	Puerto Ayacucho, Amazonas	VE	HQ290952	—	—	—
UHE147	*Anomaloglossus baeobatrachus*	-0.6455	-52.5005	UHE Santo Antonio	AP	**KY510282**	—	—	—
UTAA56469	*Anomaloglossus* sp. “Brownsberg”	4.9365	-55.1948	Brownsberg	SR	DQ502249	—	—	—
UTAA56478	*Allobates femoralis*	2.1100	-56.1700	Sipaliwini	SR	—	—	—	DQ503385
UTAA56709	*Anomaloglossus* sp. “Thomasing”	5.7423	-60.2991	Mount Thomasing	GY	DQ502253	—	—	—
UTAA56710	*Anomaloglossus* sp. “Thomasing”	5.7423	-60.2991	Mount Thomasing	GY	DQ502254	—	—	—
VUB3570	*Anomaloglossus baeobatrachus*	5.3833	-53.6500	Angoulême	FG	JQ742126	—	—	—
VUB3733	*Anomaloglossus rufulus*	5.3000	-62.1667	Churi Tepui	VE	JQ742101	—	—	—
VUB3734	*Anomaloglossus tepuyensis*	5.8528	-62.5542	Auyan-tepui	VE	JQ742104	—	—	—
VUB3735	*Anomaloglossus wothuja*	5.3746	-67.5704		VE	JQ742102	—	—	—
VUB3736	*Anomaloglossus wothuja*	4.9380	-67.3112	Cerro Sipapo	VE	JQ742103	—	—	—

Appendix B

16S pairwise distances

	sur1	sur2	sur3	sur4	sur5	deg	Ito	nor	ste	api	Aca	Bak	Bro	Par	leo	bae	sp1	Mit	bee	kai	pra	spB	ror	ruf	tep	meg	spA	spC	wot	tam
sur1	**0.013**																													
sur2	0.049	**0.002**																												
sur3	0.031	0.042	**0.002**																											
sur4	0.027	0.044	0.033	**0.004**																										
sur5	0.064	0.058	0.056	0.058	**0.000**																									
deg	0.078	0.091	0.075	0.076	0.07	**0.005**																								
Ito	0.079	0.09	0.08	0.075	0.066	0.023	**0.004**																							
nor	0.073	0.083	0.068	0.07	0.074	0.019	0.026	**0.003**																						
ste	0.111	0.111	0.114	0.101	0.101	0.103	0.112	0.107	**0.005**																					
api	0.115	0.107	0.113	0.104	0.093	0.127	0.111	0.121	0.103	**0.000**																				
Aca	0.102	0.106	0.104	0.081	0.09	0.105	0.098	0.11	0.093	0.088	**0.002**																			
Bak	0.109	0.107	0.115	0.096	0.098	0.117	0.104	0.117	0.099	0.073	0.068	**0.000**																		
Bro	0.108	0.11	0.111	0.092	0.099	0.113	0.101	0.113	0.085	0.073	0.076	0.066	**0.001**																	
Par	0.121	0.122	0.125	0.109	0.106	0.115	0.098	0.115	0.098	0.077	0.067	0.06	0.039	**0.001**																
leo	0.106	0.12	0.112	0.093	0.094	0.11	0.102	0.108	0.095	0.091	0.086	0.091	0.092	0.095	**0.001**															
bae	0.102	0.114	0.108	0.087	0.096	0.103	0.099	0.105	0.093	0.085	0.076	0.079	0.086	0.091	0.022	**0.003**														
sp1	0.098	0.109	0.104	0.083	0.094	0.103	0.106	0.102	0.084	0.087	0.07	0.082	0.077	0.088	0.034	0.029	**0.008**													
Mit	0.101	0.114	0.107	0.086	0.098	0.113	0.112	0.114	0.092	0.09	0.096	0.091	0.087	0.088	0.036	0.034	0.042	**0.002**												
bee	0.113	0.113	0.113	0.101	0.086	0.096	0.093	0.1	0.102	0.096	0.088	0.081	0.076	0.074	0.097	0.087	0.09	0.093	**0.000**											
kai	0.119	0.122	0.12	0.111	0.097	0.102	0.104	0.109	0.105	0.097	0.093	0.087	0.086	0.083	0.09	0.079	0.078	0.081	0.041	**0.006**										
pra	0.093	0.098	0.093	0.082	0.087	0.097	0.091	0.097	0.102	0.101	0.082	0.083	0.086	0.078	0.096	0.089	0.079	0.095	0.039	0.056	**0.002**									
spB	0.11	0.115	0.113	0.1	0.081	0.1	0.101	0.104	0.108	0.101	0.101	0.091	0.087	0.083	0.107	0.1	0.097	0.1	0.047	0.053	0.059	**0.008**								
ror	0.104	0.114	0.104	0.097	0.087	0.09	0.092	0.094	0.107	0.11	0.089	0.087	0.079	0.077	0.1	0.092	0.093	0.097	0.024	0.042	0.044	0.048	**0.001**							
ruf	0.111	0.124	0.116	0.105	0.095	0.098	0.097	0.095	0.102	0.106	0.101	0.081	0.092	0.089	0.097	0.096	0.084	0.09	0.053	0.074	0.056	0.064	0.048	**0.005**						
tep	0.109	0.111	0.116	0.095	0.098	0.118	0.106	0.115	0.096	0.095	0.081	0.083	0.085	0.086	0.091	0.086	0.086	0.085	0.068	0.093	0.065	0.09	0.077	0.065	**N/A**					
meg	0.113	0.114	0.121	0.1	0.095	0.1	0.091	0.099	0.094	0.101	0.087	0.085	0.087	0.086	0.086	0.082	0.086	0.087	0.077	0.088	0.087	0.092	0.076	0.079	0.082	**0.006**				
spA	0.122	0.122	0.127	0.108	0.101	0.107	0.104	0.113	0.099	0.112	0.081	0.094	0.084	0.082	0.1	0.09	0.095	0.093	0.08	0.092	0.091	0.092	0.081	0.092	0.091	0.032	**0.002**			
spC	0.118	0.119	0.122	0.105	0.098	0.096	0.086	0.095	0.099	0.109	0.085	0.092	0.092	0.084	0.092	0.092	0.09	0.096	0.074	0.092	0.086	0.093	0.073	0.077	0.086	0.014	0.037	**0.002**		
wot	0.136	0.138	0.143	0.119	0.122	0.126	0.125	0.129	0.116	0.113	0.117	0.111	0.093	0.105	0.116	0.113	0.113	0.117	0.088	0.111	0.103	0.105	0.097	0.106	0.115	0.085	0.083	0.095	**0.000**	
tam	0.144	0.155	0.152	0.138	0.132	0.139	0.133	0.142	0.108	0.128	0.12	0.116	0.098	0.109	0.121	0.109	0.101	0.113	0.093	0.112	0.097	0.103	0.098	0.103	0.12	0.111	0.118	0.11	0.134	**N/A**

Table S4. Mean intra- (bold) and inter-specific variability (p-distances) of the 16S marker in the candidate species. N/A: non applicable (only one sample available). sur1 = *Anomaloglossus surinamensis* 1; sur2 = *Anomaloglossus surinamensis* 2; sur3 = *Anomaloglossus surinamensis* 3; sur4 = *Anomaloglossus surinamensis* 4; sur5 = *Anomaloglossus surinamensis* 5; deg = *Anomaloglossus degranvillei*; Ito = *Anomaloglossus* sp. “Itoupé”; nor = *Anomaloglossus* sp. “north FG”; ste = *Anomaloglossus stepheni*; api = *Anomaloglossus apiau*; Aca = *Anomaloglossus* sp. “Acari”; Bak = *Anomaloglossus* sp. “Bakhuis”; Bro = *Anomaloglossus* sp. “Brownsberg”; Par = *Anomaloglossus* sp. “Parú”; leo = *Anomaloglossus leopardus*; bae = *Anomaloglossus baeobatrachus*; sp1 = *Anomaloglossus* sp. 1; Mit = *Anomaloglossus* sp. “Mitaraka”; bee = *Anomaloglossus beebei*; kai = *Anomaloglossus kaiei*; pra = *Anomaloglossus praderioi*; spB = = *Anomaloglossus* sp. B; ror = *Anomaloglossus roraima*; ruf = *Anomaloglossus rufulus*; tep = *Anomaloglossus tepuyensis*; meg = *Anomaloglossus megacephalus*; spA = *Anomaloglossus* sp. A; spB = *Anomaloglossus* sp. B; wot = *Anomaloglossus wothuja*; tam = *Anomaloglossus tamacuarensis*.

Appendix C

NuDNA pairwise distances

Table S5. Intra- (bold) and inter-specific divergence (p-distances) of TYR, POMC, and RAG1 grouped loci in the integrative species delimitation solution. N/A indicates non-applicable (only one sample). sur1 = *Anomaloglossus surinamensis* 1; sur2 = *Anomaloglossus surinamensis* 2; sur5 = *Anomaloglossus surinamensis* 5; nor = *Anomaloglossus* sp. "north FG"; deg = *Anomaloglossus degranvillei*; ste = *Anomaloglossus stepheni*; api = *Anomaloglossus apiau*; Bak = *Anomaloglossus* sp. "Bakhuis"; Bro = *Anomaloglossus* sp. "Brownsberg"; leo = *Anomaloglossus leopardus*; Mit = *Anomaloglossus* sp. "Mitaraka"; bae = *Anomaloglossus baeobatrachus*; pra = *Anomaloglossus praderioi*; kai = *Anomaloglossus kaiei*; bee = *Anomaloglossus beebei*; ruf = *Anomaloglossus rufulus*; tep = *Anomaloglossus tepuyensis*; meg = *Anomaloglossus megacephalus*; wot = *Anomaloglossus wothuja*.

	sur1	sur2	sur5	nor	deg	ste	api	Bak	Bro	leo	Mit	bae	pra	kai	bee	ruf	tep	meg	wot
sur1	**0.003**																		
sur2	0.006	**N/A**																	
sur5	0.009	0.012	**0.003**																
nor	0.021	0.025	0.021	**N/A**															
deg	0.021	0.024	0.02	0.003	**0.000**														
ste	0.041	0.043	0.041	0.04	0.04	**0.001**													
api	0.035	0.039	0.036	0.036	0.036	0.03	**N/A**												
Bak	0.039	0.043	0.039	0.039	0.039	0.034	0.028	**N/A**											
Bro	0.038	0.041	0.037	0.038	0.037	0.031	0.026	0.007	**N/A**										
leo	0.037	0.043	0.04	0.04	0.039	0.035	0.029	0.018	0.018	**N/A**									
Mit	0.034	0.037	0.035	0.034	0.034	0.029	0.024	0.014	0.013	0.009	**0.000**								
bae	0.036	0.04	0.037	0.037	0.036	0.034	0.027	0.017	0.016	0.012	0.004	**0.002**							
pra	0.027	0.029	0.027	0.026	0.027	0.031	0.028	0.033	0.029	0.027	0.028	0.03	**N/A**						
kai	0.031	0.034	0.031	0.032	0.032	0.035	0.029	0.032	0.03	0.028	0.027	0.03	0.008	**N/A**					
bee	0.036	0.037	0.035	0.034	0.034	0.035	0.032	0.036	0.031	0.032	0.032	0.034	0.014	0.009	**N/A**				
ruf	0.031	0.035	0.033	0.034	0.034	0.036	0.032	0.037	0.035	0.034	0.035	0.035	0.021	0.015	0.022	**N/A**			
tep	0.032	0.036	0.034	0.034	0.034	0.036	0.032	0.034	0.032	0.031	0.034	0.033	0.022	0.017	0.023	0.018	**N/A**		
meg	0.03	0.035	0.034	0.037	0.036	0.038	0.032	0.035	0.034	0.036	0.035	0.039	0.02	0.021	0.023	0.027	0.027	**N/A**	
wot	0.038	0.041	0.038	0.041	0.04	0.04	0.036	0.039	0.035	0.036	0.034	0.038	0.026	0.029	0.032	0.035	0.034	0.03	**N/A**

Appendix D

Samples of Hyloidea

Family	Species	Field number	Locality	Country	Lat	Lon
Alsodidae	*Eupsophus roseus*	JN1	Valdivia	Chile	?	?
Aromobatidae	*Allobates femoralis*	AF3224	Trois Paletuviers	French Guiana	4.0545	-51.6770
Aromobatidae	*Allobates olfersioides*	MTR16435	Itacare	BA, Brazil	-14.3655	-39.1086
Aromobatidae	*Anomaloglossus apiau*	MTR23223	Serra da Maroquinha	RR, Brazil	2.4216	-61.4129
Aromobatidae	*Anomaloglossus baeobatrachus*	AF2092	RN2	French Guiana	4.0328	-51.991
Aromobatidae	*Anomaloglossus baeobatrachus*	AF2102	RN2 corridor 5	French Guiana	4.0334	-51.99
Aromobatidae	*Anomaloglossus baeobatrachus*	AF2590	Saint-Eugène	French Guiana	4.8216	-53.0676
Aromobatidae	*Anomaloglossus baeobatrachus*	AF3032	Memora D	AP, Brazil	3.3129	-52.1803
Aromobatidae	*Anomaloglossus baeobatrachus*	MTR24258	Lourenço	AP, Brazil	2.3215	-51.6108
Aromobatidae	*Anomaloglossus baeobatrachus*	OPC26	Oiapoque	AP, Brazil	3.8355	-51.8333
Aromobatidae	*Anomaloglossus beebei*	PK0933	Kaieteur	Guyana	5.2686	-59.7686
Aromobatidae	*Anomaloglossus degranvillei*	PG601	Atachi-Bakka	French Guiana	3.5454	-53.9068

Aromobatidae	*Anomaloglossus kaiei* PK1367	Kaieteur	Guyana	5.1292	-59.4133
Aromobatidae	*Anomaloglossus leopardus* AF2039	Apalagadi	Suriname	2.1781	-56.0852
Aromobatidae	*Anomaloglossus megacephalus* PK1881	Maringma-tepui	Guyana	5.2097	-60.5662
Aromobatidae	*Anomaloglossus praderioi* PK1991	Maringma-tepui	Guyana	5.2044	-60.5775
Aromobatidae	*Anomaloglossus roraima* PK2046	Maringma-tepui	Guyana	5.2163	-60.5847
Aromobatidae	*Anomaloglossus rufulus* 214	Churi-tepui	Venezuela	5.2595	-62.0595
Aromobatidae	*Anomaloglossus* sp. 'Acari' MPEG30212	Acari	PA, Brazil	1.2854	-58.6959
Aromobatidae	*Anomaloglossus* sp. 'Bakhuis' AF3426	Bakhuis	Suriname	4.72462	-56.7638
Aromobatidae	*Anomaloglossus* sp. 'Brownsberg' BPN0850	Brownsberg	Suriname	4.9365	-55.1948
Aromobatidae	*Anomaloglossus* sp. 'Itoupé' PG660	Itoupé	French Guiana	3.025	-53.08
Aromobatidae	*Anomaloglossus* sp. 'Mitaraka' PG302	Haut Marwini	French Guiana	2.6152	-54.0327
Aromobatidae	*Anomaloglossus* sp. 'north FG' AF0932	Kaw-Patawa	French Guiana	4.54	-52.1527
Aromobatidae	*Anomaloglossus stepheni* AF2045	Apalagadi	Suriname	2.1781	56.0852
Aromobatidae	*Anomaloglossus surinamensis* AF0585	Carbet Saint-Elie	French Guiana	5.3415	-53.0388
Aromobatidae	*Anomaloglossus surinamensis* AF2456	Nassau	Suriname	4.8172	-54.5909
Aromobatidae	*Anomaloglossus surinamensis* AF3340	Bakhuis	Suriname	4.7246	-56.7638
Aromobatidae	*Anomaloglossus tamacuarensis* MNRJ38049	Pico Tamacuari	AM, Brazil	1.2092	-64.7483
Aromobatidae	*Anomaloglossus tepuyensis* 216	Auyan-tepui	Venezuela	5.7690	-62.5340

Aromobatidae	*Anomaloglossus wothuja*	FS5627	Tobogan de la Selva	Venezuela	5.3855	-67.6145
Aromobatidae	*Mannophryne collaris*	FS5523	?	?	?	?
Aromobatidae	*Rheobates palmatus*	RHEOPALM	?	?	?	?
Batrachylidae	*Batrachyla taeniata*	JN2	Valdivia	Chile	?	?
Bufonidae	*Amazophrynella* sp.	AF2713	Alikéné	French Guiana	3.2187	-52.3964
Cycloramphidae	*Cycloramphus eleuthero-dactylus*	AF1746	Estacio Biologica de Boracéia	SP, Brazil	-23.6330	-45.5330
Dendrobatidae	*Ameerega hahneli*	AF2673	Alikéné	French Guiana	3.2081	-52.4024
Hylodidae	*Crossodactylus* sp.	H155	?	?	?	?
Leptodactylidae	*Rupirana cardosoi*	JC1146	Mucuga	BA, Brazil	-12.9708	-41.3559

Table D.1 – List of all samples for which mitogenomes were sequenced through HiSeq 2500 sequencing technology (Illumina).

Table D.2: List of all samples used to produce the complete matrix for phylogenetic analyses for all loci, with GenBank accession numbers. Newly produced sequences deposited in GenBank appear in bold. The newly produced sequences not deposited in GenBank begin with "XXX".

mitogenome	**28S**	**TYR**	**POMC**	**RAG1-1**	**RAG1-2**
Allobates femoralis XXXAF3224	*A. femoralis* DQ283465	*A. femoralis* DQ503156	*A. femoralis* HQ290831	*A. granti* JX298193	*A. femoralis* DQ503327
Allobates olfersioides XXXMTR16435	*A. olfersioides* XXXMTR16435	*A. olfersioides* XXXMTR16435	–	*A. olfersioides* XXXMTR16435	*A. olfersioides* XXXMTR16435
Alsodes gargola JX564852	*A. gargola* AY844197	–	*A. neuquensis* KP295561	*A. gargola* JX204069	*A. gargola* JX204069
Amazophrynella sp. XXXAF2713	*A.* sp. XXXAF2014	*A.* sp. JX298240	*A.* cf. *minuta* AY819081	*A.* cf. *minuta* DQ158346	*A.* cf. *minuta* DQ503337
Ameerega hahneli XXXAF2673	*A.* hahneli XXXAF2673	*A. hahneli* DQ503174	*A. hahneli* XXXAF2673	*A. hahneli* XXXAF2673	*A. hahneli* XXXAF2673
Anomaloglossus apiau XXXMTR23223	*A. apiau* XXXMTR23223	*A. apiau* **KY549555**	*A. apiau* **KY549524**	*A. apiau* **KY549483**	*A. apiau* **KY549483**
Anomaloglossus baeobatrachus XXXAF2092	*A. baeobatrachus* XXXAF2092	*A. baeobatrachus* **KY549536**	*A. baeobatrachus* **KY549496**	*A. baeobatrachus* **KY549454**	*A. baeobatrachus* **KY549454**
Anomaloglossus baeobatrachus XXXAF2102	*A. baeobatrachus* XXXAF2102	*A. baeobatrachus* **KY549537**	*A. baeobatrachus* **KY549497**	*A. baeobatrachus* **KY549455**	*A. baeobatrachus* **KY549455**
Anomaloglossus baeobatrachus **KU958559**	*A. baeobatrachus* XXXAF2590	*A. baeobatrachus* **KY549544**	*A. baeobatrachus* **KY549504**	*A. baeobatrachus* **KY549462**	*A. baeobatrachus* **KY549462**
Anomaloglossus baeobatrachus XXXAF3032	*A. baeobatrachus* XXXAF3032	*A. baeobatrachus* XXXAF3032	*A. baeobatrachus* XXXAF3032	*A. baeobatrachus* XXXAF3032	–
Anomaloglossus baeobatrachus XXXMTR24258	*A. baeobatrachus* XXXMTR24258	*A. baeobatrachus* XXXMTR24258	–	*A. baeobatrachus* XXXMTR24258	*A. baeobatrachus* XXXMTR24258
Anomaloglossus baeobatrachus XXXOPC26	*A. baeobatrachus* XXXOPC26	*A. baeobatrachus* **KY549556**	*A. baeobatrachus* **KY549525**	*A. baeobatrachus* **KY549484**	*A. baeobatrachus* **KY549484**
Anomaloglossus beebei XXXPK0933	*A. beebei* XXXPK0933	*A. beebei* JN691804	*A. beebei* XXXPK0933	–	*A. beebei* XXXPK0933
Anomaloglossus degranvillei XXXPG601	–	*A. degranvillei* **KY549557**	*A. degranvillei* **KY549526**	*A. degranvillei* **KY549485**	*A. degranvillei* **KY549485**
Anomaloglossus kaiei XXXPK1367	*A. kaiei* XXXPK1367	*A. kaiei* JN691805	*A. kaiei* **KY549528**	*A. kaiei* **KY549487**	*A. kaiei* **KY549487**
Anomaloglossus leopardus XXXAF2039	*A. leopardus* XXXAF2039	*A. leopardus* XXXAF2039	*A. leopardus* XXXAF2039	*A. leopardus* XXXAF2039	*A. leopardus* XXXAF2039
Anomaloglossus megacephalus XXXPK1881	*A. megacephalus* XXXPK1881	*A. megacephalus* JN691802	*A. megacephalus* **KY549529**	*A. megacephalus* **KY549488**	*A. megacephalus* **KY549488**
Anomaloglossus praderioi XXXPK1991	*A. praderioi* XXXPK1991	–	*A. praderioi* **KY549530**	*A. praderioi* **KY549489**	*A. praderioi* **KY549489**
Anomaloglossus roraima XXXPK2046	*A. roraima* XXXPK2046	–	–	–	*A. roraima* DQ503395
Anomaloglossus rufulus XXX214	*A. rufulus* XXX214	–	*A. rufulus* **KY549491**	*A. rufulus* **KY549447**	–
Anomaloglossus sp. 'Acari' XXXMPEG30212	*A.* sp. 'Acari' XXXMPEG30212	–	–	–	–
Anomaloglossus sp. 'Bakhuis' XXXAF3426	*A.* sp. 'Bakhuis' XXXAF3426	*A.* sp. 'Bakhuis' **KY549552**	*A.* sp. 'Bakhuis' **KY549513**	*A.* sp. 'Bakhuis' **KY549471**	*A.* sp. 'Bakhuis' **KY549471**
Anomaloglossus sp. 'Brownsberg' XXXBPN0850	*A.* sp. 'Brownsberg' XXXBPN0850	*A.* sp. 'Brownsberg' JN691716	*A.* sp. 'Brownsberg' KY549514	*A.* sp. 'Brownsberg' KY549472	*A.* sp. 'Brownsberg' KY549472
Anomaloglossus sp. 'Itoupé' XXXPG660	*A.* sp. 'Itoupé' XXXPG660	–	–	–	–

Anomaloglossus sp. 'Mitaraka' XXXPG302	*A.* sp. 'Mitaraka' XXXPG302	*A. baeobatrachus* JN691704	–	–	–
Anomaloglossus sp. 'north FG' XXXAF0932	*A.* sp. 'north FG' XXXAF0932	*A.* sp. 'north FG' **KY549533**	*A.* sp. 'north FG' XXXAF0932	*A.* sp. 'north FG' XXXAF0932	*A.* sp. 'north FG' XXXAF0932
Anomaloglossus stepheni XXXAF2045	*A. stepheni* XXXAF2045	*A. stepheni* **KY549535**	*A. stepheni* **KY549495**	*A. stepheni* **KY549453**	*A. stepheni* **KY549453**
Anomaloglossus surinamensis XXXAF0585	*A. surinamensis* XXXAF0585	*A. surinamensis* XXXAF0585	–	*A. surinamensis* XXXAF0585	*A. surinamensis* XXXAF0585
Anomaloglossus surinamensis XXXAF2456	*A. surinamensis* XXXAF2456	*A. surinamensis* XXXAF2456	*A. surinamensis* XXXAF2456	*A. surinamensis* XXXAF2456	–
Anomaloglossus surinamensis XXXAF3340	*A. surinamensis* XXXAF3340	*A. surinamensis* **KY549550**	*A. surinamensis* **KY549511**	*A. surinamensis* **KY549469**	*A. surinamensis* **KY549469**
Anomaloglossus tamacuarensis XXXMNRJ38049	–	–	–	–	–
Anomaloglossus tepuyensis XXX216	*A. tepuyensis* XXX216	–	*A. tepuyensis* **KY549492**	*A. tepuyensis* **KY549448**	–
Anomaloglossus wothuja XXXFS5627	*A. wothuja* XXXFS5627	*A. wothuja* XXXFS5627	*A. wothuja* XXXFS5627	*A. wothuja* XXXFS5627	*A. wothuja* XXXFS5627
Aromobates aff. alboguttatus HQ290953	*A. nocturnus* DQ502998	*A. aff. alboguttatus* HQ290893	*A. aff. alboguttatus* HQ290833	–	*A. saltuensis* DQ503406
Batrachyla taeniata XXXJN2	*B. taeniata* XXXJN2	*B. leptopus* AY844028	*B. leptopus* KP295563	*B. taeniata* JN2	*B. leptopus* AY844369
Bufo gargarizans DQ275350	*B. gargarizans* DQ283599	*B. bufo* FM864217	*B. gargarizans* KU183159	*B. bufo* AY583336	*B. bufo* AY583336
Callimedusa tomopterna JX564887	*C. tomopterna* AY844328	*C. nordestina* KM387556	*C. tomopterna* GQ366068	*C. tomopterna* EF174319	*C. tomopterna* AY844497
Calyptocephalella gayi JF703228	–	*C. gayi* JX298244	*C. gayi* AY819090	*C. gayi* AY583337	*C. gayi* AY583337
Ceratophrys ornata JX564858	*C. cranwelli* AY844207	*C. ornata* KP295675	*C. ornata* AY819091	*C. cornuta* DQ679269	*C. cornuta* DQ679269
Craugastor augusti JX564870	*C. augusti* DQ283627	*C. podiciferus* EF493481	*C. podiciferus* GQ345258	*C. podiciferus* GQ345277	*C. podiciferus* GQ345292
Crossodactylus sp. XXXH155	*C. schmidti* AY844210	*C. schmidti* AY844031	*C. caramaschii* KC604072	*C. caramaschii* KJ961589	*C. caramaschii* KJ961589
Cycloramphus eleutherodactylus XXXAF1746	*C. boraceiensis* DQ283498	*C. boraceiensis* DQ282924	*C. euletherodactylus* AF1746	*C. acangatan* HQ634170	*C. acangatan* FJ685703
Dendrobates auratus JX564862	*D. auratus* AY844211	*D. auratus* HQ290917	*D. auratus* HQ290857	*D. auratus* AY364214	*D. auratus* AY364214
Eleutherodactylus atkinsi JX564864	*E. nitidus* DQ283647	*E. cooki* EF493455	*E. cooki* HQ831999	*E. johnstonei* JX298190	*E. johnstonei* JX298190
Eupsophus roseus XXXJN1	*E. calcaratus* AY844214	*E. calcaratus* AY844036	*E. roseus* KC604074	*E. roseus* KC604032	*E. roseus* KC604032
Gastrotheca pseustes JX564866	*Hemiphractus bubalus* GQ345134	*G. cornuta* AY844040	*G. pseustes* KC844986	*G. cornuta* DQ679280	*G.* cf. *marsupiata* AY844380
Hyalinobatrachium fleischmanni JX564869	*H. fleischmanni* DQ283756	*Cochranella bejaranoi* AY844029	*H. aff. mondolfii* EU663211	*Vitreorana uranoscopa* JX298194	*H. valerioi* EU663519
Hyla annectans KM271781	*H. arborea* AY844221	*H. annectans* AY844045	*H. annectans* DQ055786	*H. arborea* KF587690	*H. arborea* AY844389
Hyla japonica AB303949	*H. japonica* AY844255	*H. japonica* AY844078	*H. japonica* KP742515	*H. japonica* FJ227068	*H. japonica* AY844420
Hylodes meridionalis KT221614	*H. phyllodes* DQ503009	*H. phyllodes* DQ282923	*H. nasus* GQ345272	*H. phyloodes* KC604006	*H. phyloodes* DQ503367
Hyloxalus yasuni KT221612	*H. bocagei* DQ502961	*H. subpunctatus* HQ290911	*H. subpunctatus* HQ290851	–	*H. subpunctatus* DQ503405
Lechriodus melanopyga JF703230	*Limnodynastes depressus* DQ283643	*L. victoriana* DQ282965	*Notaden Bennettii* AY819099	*L. melanopyga* AY583341	*L. melanopyga* AY583341

Leptodactylus melanonotus JX564873	*L. fuscus* DQ283716	*L. melanonotus* DQ347193	*L. longirostris* KC604057	*L. myersi* JX298196	*L. myersi* JX298196
Mannophryne collaris XXXFS5523	*M. collaris* XXXFS5523	*M. collaris* HQ290941	*M. collaris* XXXFS5523	*M. collaris* XXXFS5523	*M. collaris* XXXFS5523
Mannophryne trinitatis JX564878	*M.* sp. DQ503026	–	*M. trinitatis* JX036003	*M. trinitatis* GQ345274	*M. trinitatis* DQ503345
Melanophryniscus simplex KT221611	*M. klappenbachi* AY844306	*M. alipioi* KX026258	*M. klappenbachi* KP295580	*M. stelzneri* DQ158347	*M. klappenbachi* DQ503299
Nyctimystes kubori JX564879	*N. narinosus* AY844308	*N. narinosus* AY844135	*N. foricula* AY819150	*N. pulcher* Y948941	*N. kubori* AY844479
Odontophrynus occidentalis JX564880	*O. achalensis* DQ283611	*O. americanus* JX298239	*O. americanus* JX298141	*O. americanus* JX298191	*O. americanus* FJ685706
Oophaga pumilio HQ290988	*O. histrionica* DQ502982	*O. pumilio* HQ290925	*O. pumilio* HQ290865	*O. pumilio* EU325918	*O. pumilio* GQ980855
Osteocephalus taurinus JX564881	*O. taurinus* AY844313	*O. taurinus* AY844140	*O. taurinus* AY819130	*O. taurinus* EU034135	*O. taurinus* EU034135
Pleurodema thaul JX564888	*P. marmorata* GQ345144	*P. diplolister* KC604080	*P. diplolister* KC604052	*P. diplolister* HQ634173	*P. brachyops* AY844503
Pristimantis thymelensis JX564889	*P. pluvicanorus* AY844213	*P. thymelensis* EF493503	*P.* sp. JX298140	*P.* sp. KC604010	*P. curtipes* DQ679272
Rheobates palmatus XXXRHEOPALM	*R. palmatus* XXXRHEOPALM	*R. palmatus* DQ503172	*R. palmatus* XXXRHEOPALM	*R. palmatus* XXXRHEOPALM	–
Rhinella sp. KT221613	*R. marina* DQ283472	*R. margaritifera* KR012541	*R. arenarum* DQ158271	*R. arenarum* DQ158354	*R. arenarum* AY844370
Rhinoderma darwinii JX564891	*R. darwinii* DQ283654	–	*R. darwinii* KP295584	*R. darwinii* AY364222	–
Rupirana cardosoi XXXJC1146	*R. cardosoi* XXXJC1146	*R. cardosoi* KC604078	*R. cardosoi* KC604049	*R. cardosoi* KC604012	*R. cardosoi* KC604034
Telmatobius bolivianus JF703234	*T. sibiricus* AY844355	*T.* sp. DQ347182	*T. truebae* AY819097	*T. bolivianus* AY583344	*T. bolivianus* AY583344

Appendix E

BEAST partition scheme

Partition	Genes	Model
1	28S	GTR+G+I
2	POMC, RAG1, TYR	GTR+I+G
3	tRNA-Cys, tRNA-Leu1, tRNA-Lys, tRNA-Ser2, tRNA-Tyr	GTR+G+I
4	tRNA-Ala, tRNA-Arg, tRNA-Asp, tRNA-Gln, tRNA-Glu, tRNA-Gly, tRNA-His, tRNA-Ile, tRNA-Leu2, tRNA-Phe, tRNA-Pro, tRNA-Thr, tRNA-Trp, tRNA-Val	GTR+G+I
5	12S, tRNA-Asn, tRNA-Met, tRNA-Ser	GTR+G+I
6	16S	GTR+G+I
7	ATP6, ATP8, ND1, ND2, ND3, ND4, ND4L, ND5	GTR+G+I
8	rep-origin	SYM+G
9	COX1, COX2, COX3	GTR+G+I
10	ND6	TrN+G+I
11	CYTB	GTR+G+I

Table E.1 – Best-fit partition scheme recovered by PartitionFinder for possible models implemented in BEAST for mitochondrial genes, rRNA, tRNA, and nuclear genes and rRNA.

Appendix F

Phylogeny of Hyloidea

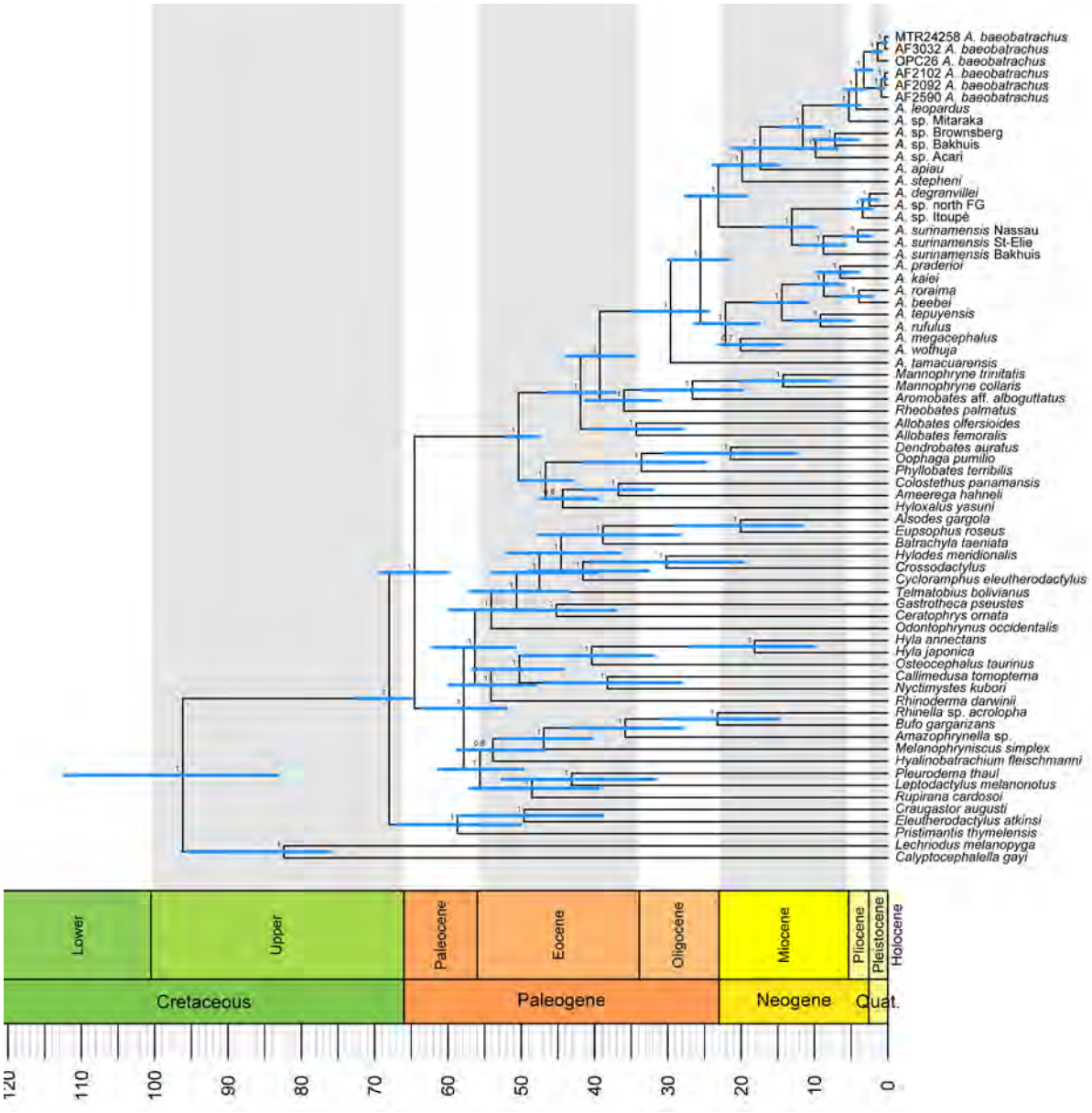

Figure F.1 – Time-calibrated phylogeny of Hyloidea as recoverded by BEAST. Posterior probabilities are indicated above nodes. 95 % time confidence intervals are indicated by blue bars. Two species of Myobatrachoidea are used as outgroup.

Appendix G

ML tree of Hyloidea

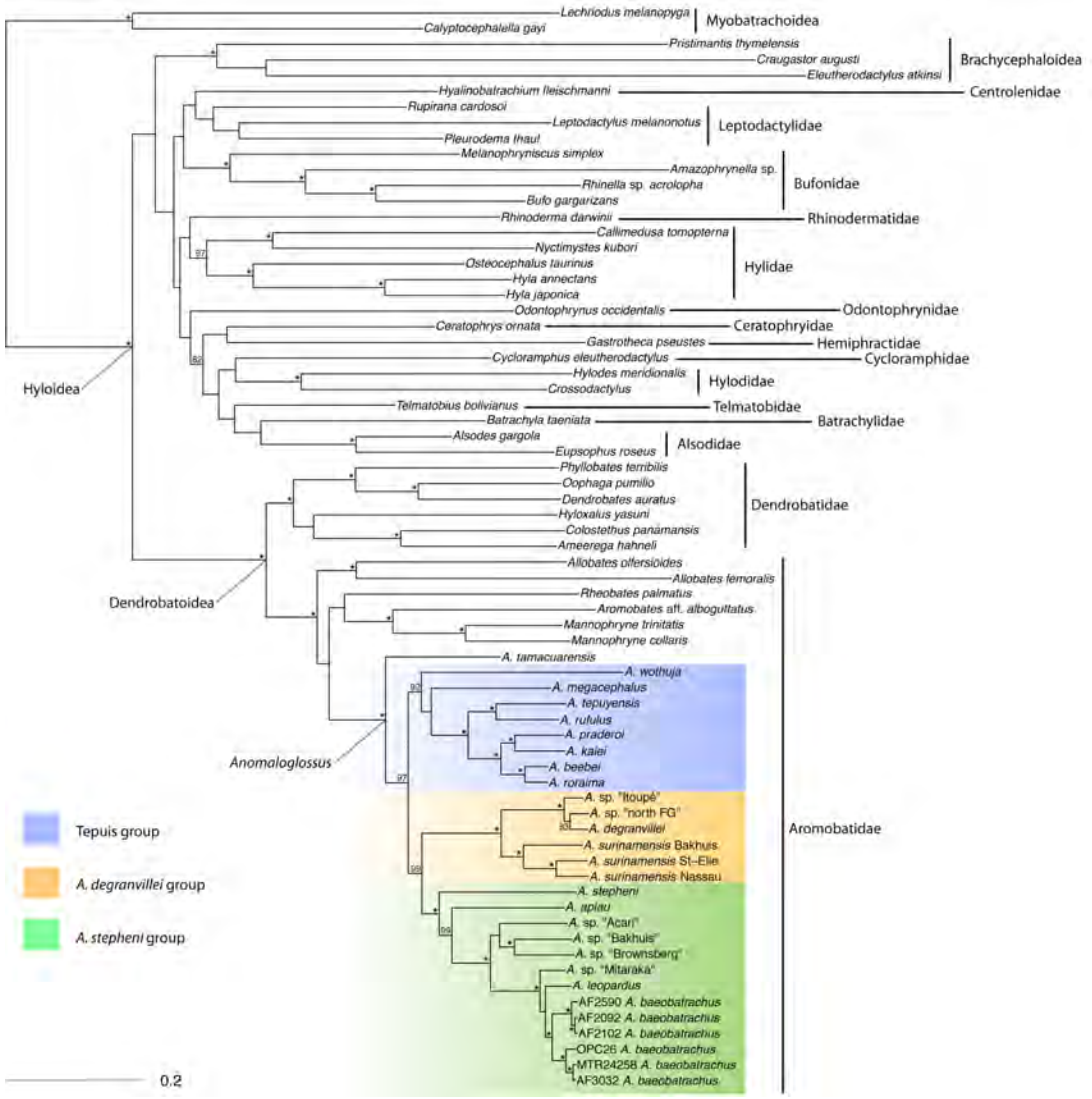

Figure G.1 – Maximum likelihood tree obtained from concatenated mitogenomes and nuDNA loci *28S*, *TYR*, *POMC*, *RAG1*. Bootstrap values are indicated above nodes (* = 100 %; not indicated when <80 %).

Appendix H

Mitogenome and nuDNA ML trees of Hyloidea

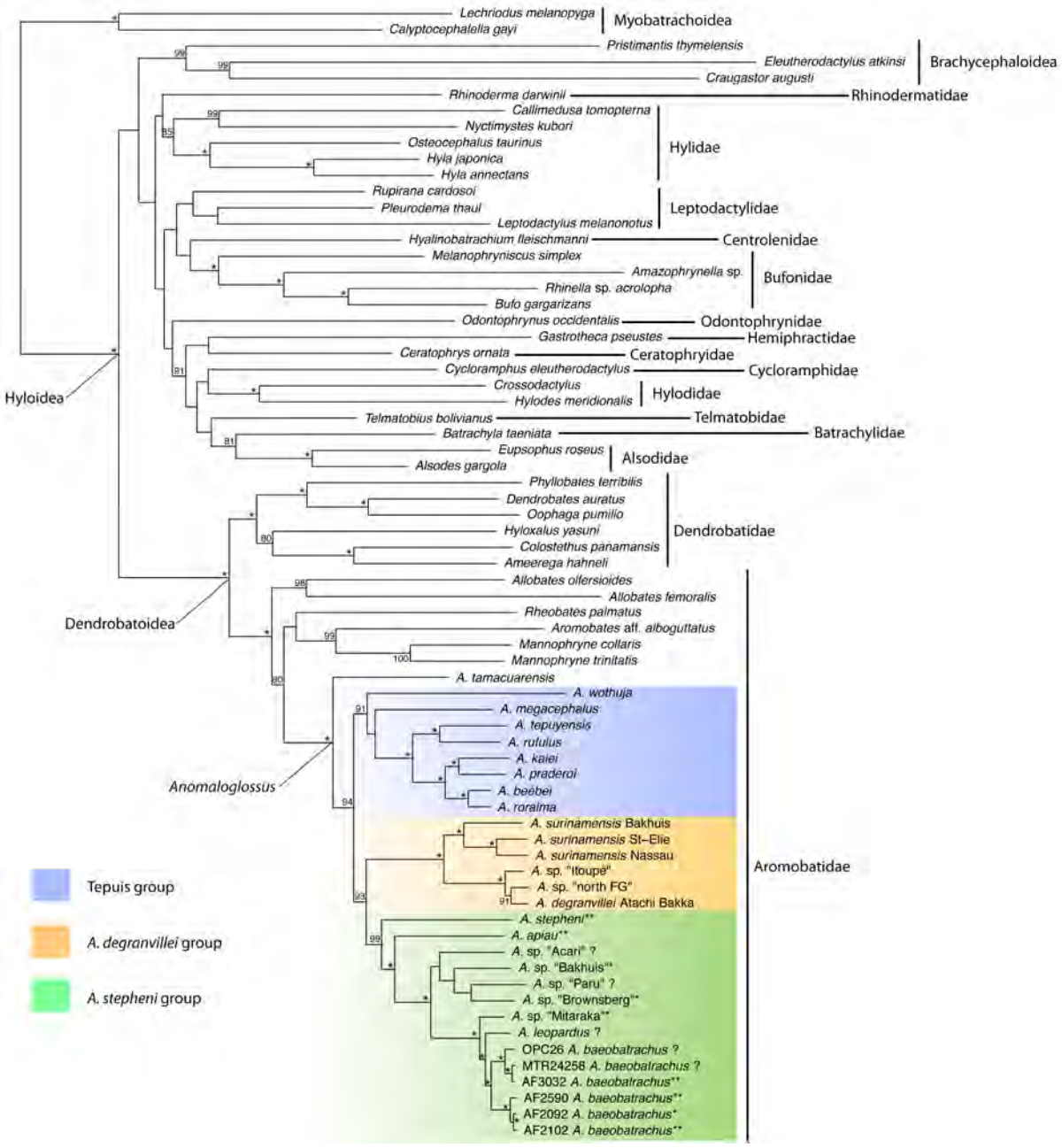

Figure H.1 – Maximum likelihood tree of Dendrobatoidea obtained from mitogenomes. Bootstrap values are indicated above nodes (* = 100 %; not indicated when <80 %).

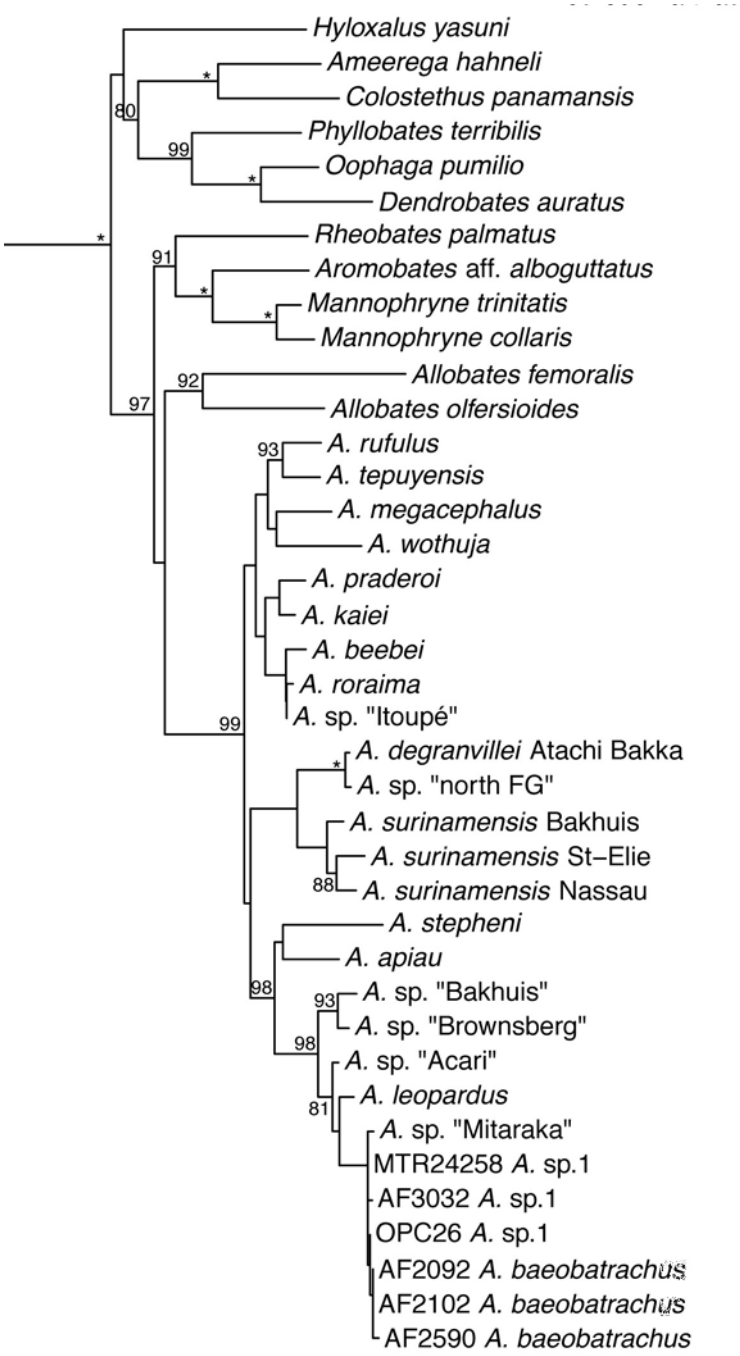

Figure H.2 – Maximum likelihood tree obtained from concatenated nuDNA fragments of *TYR*, *POMC*, *RAG1*, and *28S*. Bootstrap values are indicated above nodes (* = 100 %; not indicated when <80 %).

Appendix I

BioGeoBEARS plots

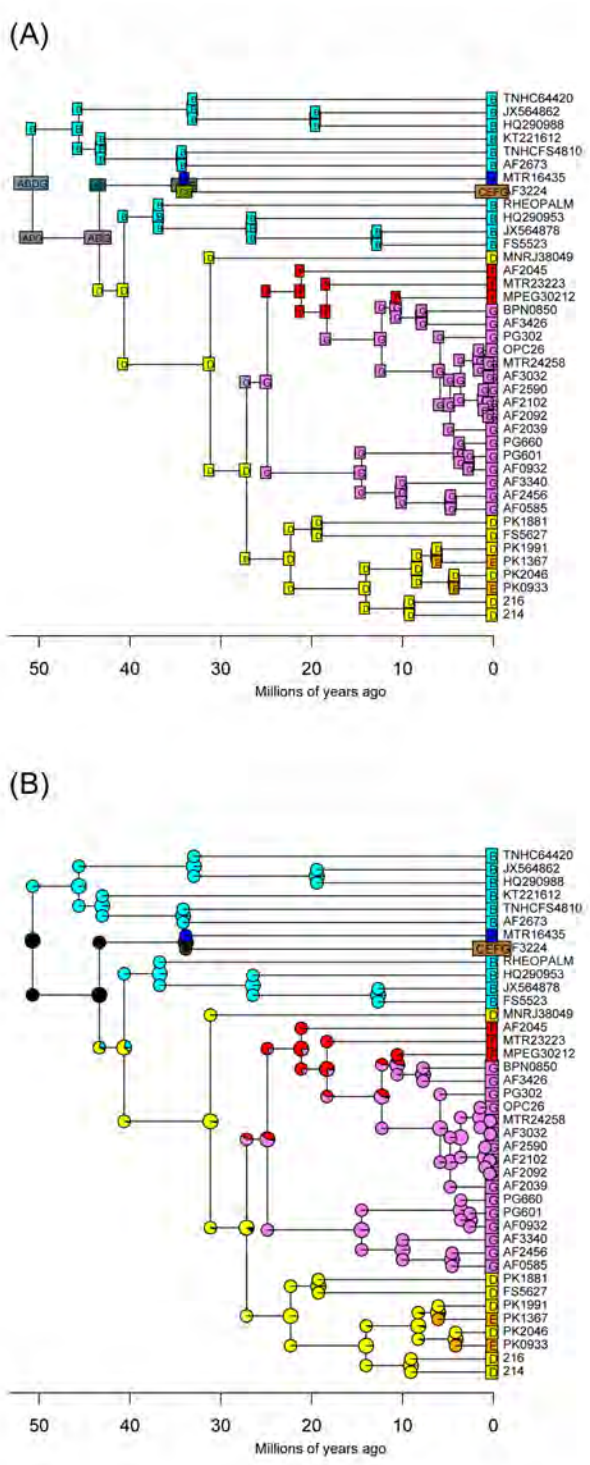

Figure I.1 – Ancestral area reconstruction of Dendrobatoidea, with the range of all Dendrobatidae constrained to "Andes", and the range of *Anomaloglossus stepheni* constrained at southern EGS. (A) DIVA+j model; (B) DIVA+j model piecharts displaying the likelihood of each ancestral area. The tree topology is derived from the BEAST analysis. Colours represent area assignation for species at tips and most probable states at each node and stems. The states at nodes represent the most probable ancestral area before speciation, whereas states at stem represent the area of the descendant lineage right after speciation. A = Mata Atlantica; B = Andes; C = Amazonia; D = Tepuis; E = Eastern Guiana Shield–West; F = Eastern Guiana Shield–South; G = Eastern Guiana Shield–East.

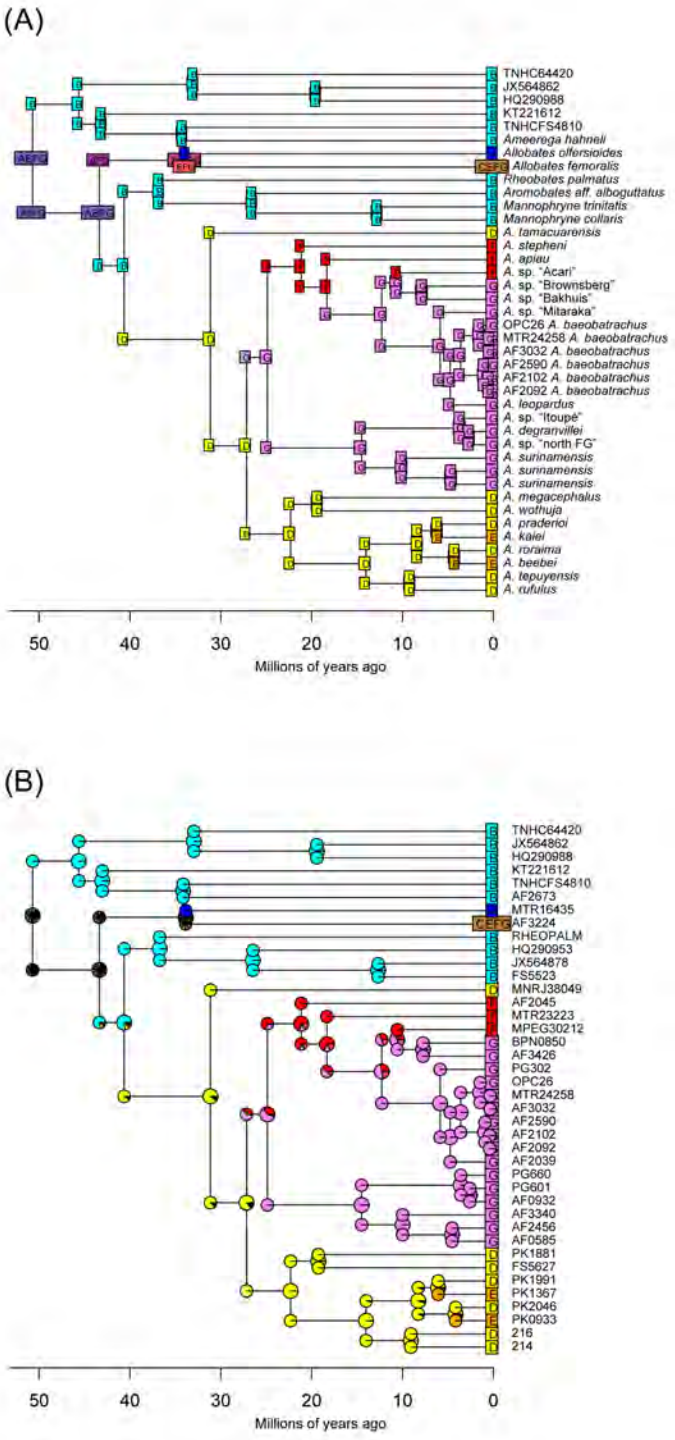

Figure I.2 – Ancestral area reconstruction of Dendrobatoidea, with the range of all Dendrobatidae constrained to "Andes", and the range of *Anomaloglossus stepheni* constrained at southern EGS. (A) DEC+j model; (B) DEC+j model piecharts displaying the likelihood of each ancestral area. The tree topology is derived from the BEAST analysis. Colours represent area assignation for species at tips and most probable states at each node and stems. The states at nodes represent the most probable ancestral area before speciation, whereas states at stem represent the area of the descendant lineage right after speciation. A = Mata Atlantica; B = Andes; C = Amazonia; D = Tepuis; E = Eastern Guiana Shield–West; F = Eastern Guiana Shield–South; G = Eastern Guiana Shield–East.

Appendix J

Ancestral traits reconstruction with unknown characters

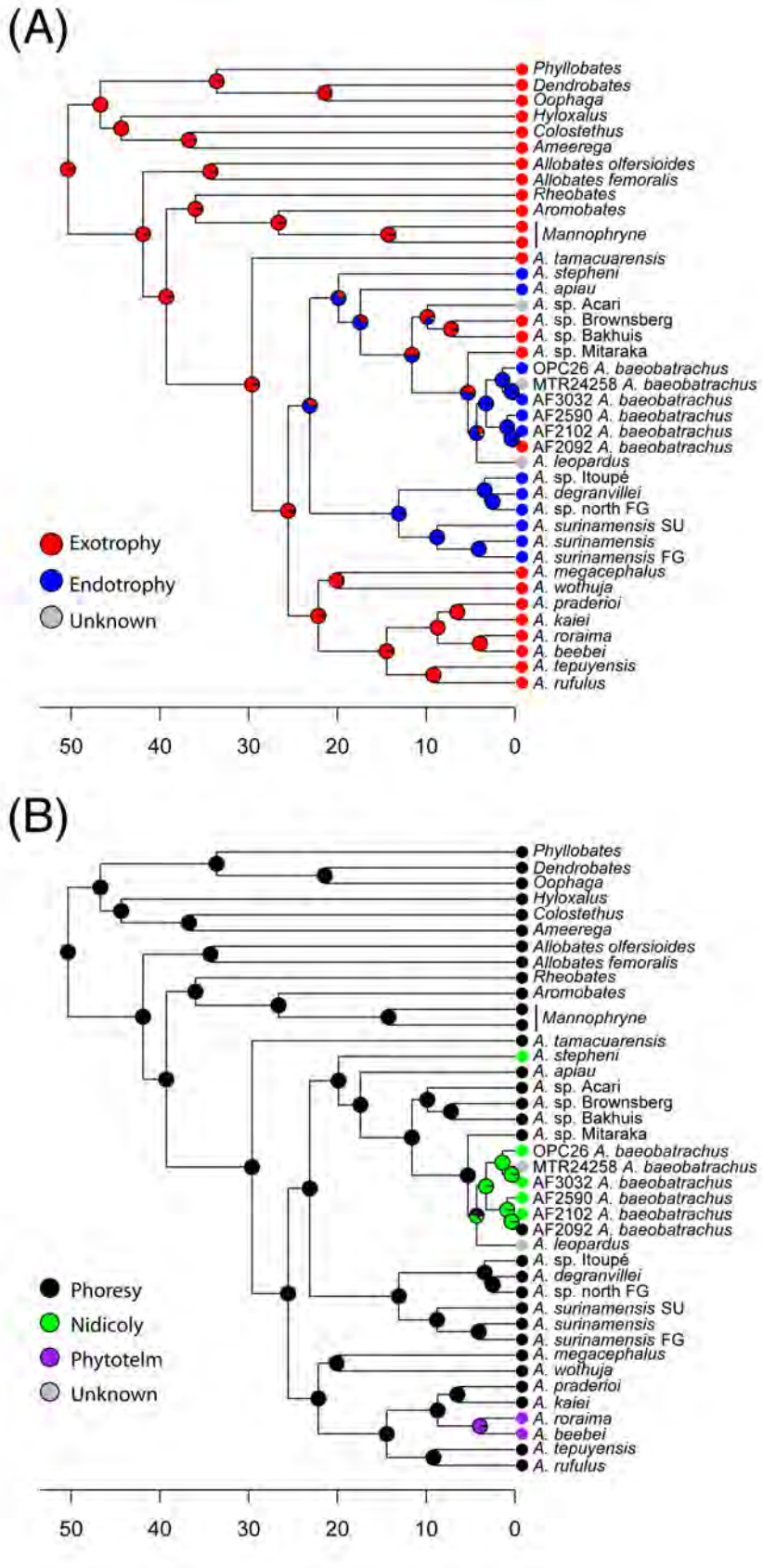

Figure J.1 – Discrete trait reconstruction based on stochastic character mapping method with an empirical estimation of Q with unknown states of characters for three terminals (A) exotrophy vs. endotrophy; (B) phoresy vs. nidicoly vs. phytotelm breeders. SU = Suriname; FG = French Guiana.

Changes	1,2	1,4	2,1	2,4	4,1	4,2
x–>y	2.44	1.26	2.41	2.05	0.45	0.25

Table J.1 – Average number of changes for traits associated with exotrophy and endotrophy. 1 = exotrophy; 2 = endotrophy; 4 = unknown

Changes	1,2	1,3	1,4	2,1	2,3	2,4	3,1	3,2	3,4	4,1	4,2	4,3
x–>y	1.89	1.10	0.68	1.16	0.01	1.41	0.08	0.04	0.00	0.07	0.15	0.01

Table J.2 – Average number of changes for traits associated with phorey, nidicoly, and phytotelm breeders. 1 = phoresy; 2 = nidicoly; 3 = phytotelm breeders, 4 = unknown

Appendix K

An overview of some *Anomaloglossus* species of the eastern Guiana Shield

Figure K.1 – Male *Anomaloglossus surinamensis* with tadpoles on its back, from the type locality of the species, Nassau Mountains, Suriname © Antoine Fouquet.

Figure K.4 – Male *Anomaloglossus baeobatrachus*, field number AF2590, from the type locality of the species, Saint-Eugène, French Guiana © Jean-Pierre Vacher.

Figure K.2 – Calling male of *Anomaloglossus stepheni*, from Apalagadi Mountain, Suriname © Jean-Pierre Vacher.

Figure K.5 – Tadpole of *Anomaloglossus stepheni* in the nest, from Sipaliwini, Suriname © Antoine Fouquet.

Figure K.3 – Male *Anomaloglossus leopardus* from Apalagadi Mountain, Suriname © Jean-Pierre Vacher.

Figure K.6 – Male *Anomaloglossus apiau* carrying tadpoles, from Serra do Apiau, Roraima, Brazil © Antoine Fouquet.

Appendix L

Published article – *The complete mitogenome of* Anomaloglossus baeobatrachus *(Amphibia: Anura: Aromobatidae)*

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MITOGENOME ANNOUNCEMENT

OPEN ACCESS

The complete mitochondrial genome of *Anomaloglossus baeobatrachus* (Amphibia: Anura: Aromobatidae)

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ABSTRACT

The complete mitogenome of the rocket frog *Anomaloglossus baeobatrachus* was sequenced using a shotgun approach on an Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA), providing the first mitogenome for this genus. The genome was 17,572 bp long and presents the typical organization found in other neobatrachian anurans. A phylogenetic analysis including *A. baeobatrachus* and all other available mitogenomes of Hyloidea provided relationships in accordance with previous phylogenetic studies.

ARTICLE HISTORY
Received 23 March 2016
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KEYWORDS
Amphibia; Aromobatidae; Guiana Shield; mitochondrial genome

Anomaloglossus baeobatrachus (Boistel & Massary, 1999) is a species of frog endemic to the eastern part of the Guiana Shield. It is currently known to occur in French Guiana, Suriname and the State of Amapá (Fouquet et al. 2012), and the State of Pará (Avila-Pires et al. 2010). The taxonomy of the genus *Anomaloglossus* is not well resolved, as several mitochondrial lineages currently associated with nominal species might in fact represent undescribed species (Fouquet et al. 2007, 2012; Kok et al. 2012). This is the case of *A. baeobatrachus* for which four distinct mitochondrial lineages have been identified (Fouquet et al. 2012). Molecular data can significantly contribute in resolving the systematics and species boundaries within this genus but available genomic data are still scarce. Here, we describe the complete mitochondrial genome of *Anomaloglossus baeobatrachus*.

A calling male of *A. baeobatrachus* was collected at Saint-Eugène, French Guiana (4°49′17.2″N; 53°04′03.4″W), the *terra typica* of the species (Boistel & Massary, personal communication). DNA was isolated from liver tissue using the Wizard Genomic extraction protocol (Promega Inc., Madison, WI). We then used 200 ng of DNA to create a DNA sequencing library at the Genopole of Toulouse (France). The library was hybridized and sequenced on a 1/24th of lane of an Illumina HiSeq 2500 flow cell (Illumina Inc., San Diego, CA). Over 24 million paired-end read of 150 bp were obtained. The mitochondrial genome was assembled using an iterative mapping strategy (Besnard et al. 2014). We obtained a circular sequence of 17,572 bp in length. The overall base composition was as follows: A (28.5%), C (27.4%), G (13.9%) and T (30.3). We annotated the mitogenome with the MITOS webserver (Bernt et al. 2013). We validated the coding regions using Geneious version 9.0.5 (Kearse et al. 2012). The annotated sequence was submitted to NCBI (accession no. KU958559).

We then used MAFFT v.7 (Katoh & Standley 2013) to align the mitogenome of *A. baeobatrachus* with all available mitochondrial genomes of Hyloidea (Nobleobatrachia), a superfamily of Neobatrachia. The gene order was fully conserved in this clade, and we conducted a maximum-likelihood phylogenetic analysis on this alignment with RAxML v. 8.2.4. (Stamatakis 2014) excluding the control region. The resulting phylogenetic tree (Figure 1) shows that *A. baeobatrachus* and *Mannophryne trinitatis*, which belong to the family Aromobatidae, form a strongly supported clade. This clade is the sister group of Dendrobatidae, which is in accordance with previous studies (Grant et al. 2006). Given several species within this genus might face decline or might already have gone extinct (Courtois et al. 2015; Fouquet et al. 2015), resolving taxonomic uncertainties is crucial to assess conservation priorities. These data, which represent the first mitogenome for the genus and the second for Aromobatidae, will serve as a reference for further studies on the taxonomy and evolution of this group of amphibians.

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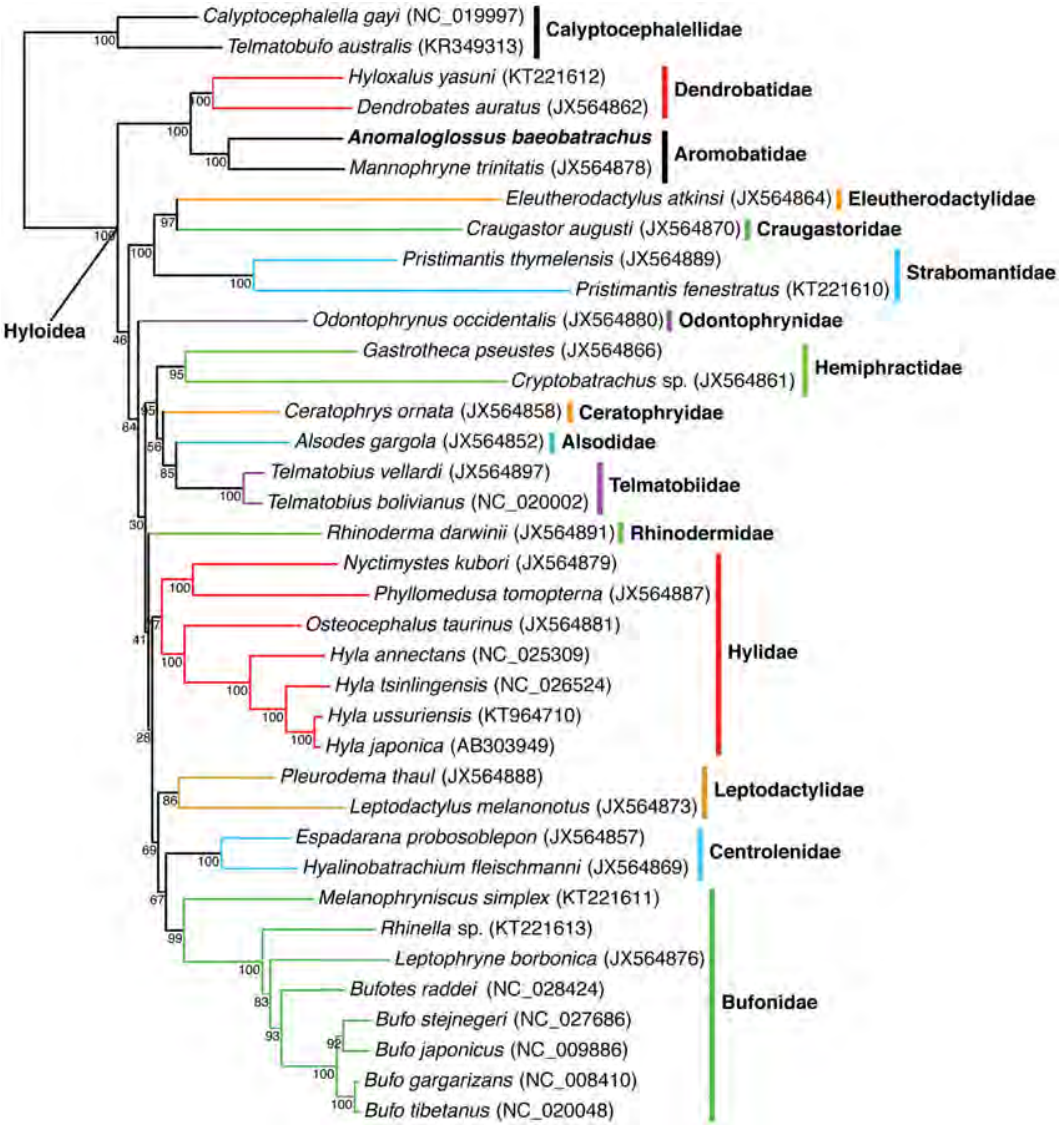

Figure 1. Maximum-likelihood phylogeny of Hyloidea inferred with a GTR + G + I model from all available mitochondrial genomes in this clade. Calyptocephalellidae was used to root the tree. The new sequence is represented in bold. The Bootstrap values (based on 1000 iterations and 100 independent maximum-likelihood searches) are indicated for each internal node.

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Disclosure statement

The authors report that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Diversification in the Guiana Shield as seen through frogs.

Abstract The Guiana Shield has been geologically stable during the Cenozoic era, exempt of the influence of the uplift of the Andes and the setting up of the Amazon basin. Is this region biogeographically homogeneous within Amazonia? What are the spatio-temporal diversification modalities within this region? To answer these questions, I explored bioregionalisation within Amazonia and the Guiana Shield based on the distribution of anuran amphibians. This approach enabled to define three bioregions in the eastern Guiana Shield and to reveal a high underestimation of endemism. Then, I studied the diversification patterns within the endemic frog genus *Anomaloglossus*. This part enabled to reveal cryptic speciation within the genus, and a biogeographic pattern composed of four areas of diversification in the Guiana Shield, with an origin of the genus in the western highlands (tepuis).

Keywords Amazonia. Guiana Shield. Diversity. Bioregionalisation. Endemism. Diversification. Biogeography. Evolution. Integrative taxonomy. Amphibians.

Auteur Jean-Pierre Vacher

Titre Diversification in the Guiana Shield as seen through frogs (*La diversification au sein du Plateau des Guyanes vue à travers le prisme des amphibiens anoures*).

Directeurs de thèse Christophe Thébaud et Antoine Fouquet

Thèse soutenue le 23 mars 2017

Résumé Le Plateau des Guyanes a été géologiquement stable au cours de l'ère Cénozoïque, exempt de l'influence de l'orogénèse des Andes et de la mise en place du bassin de l'Amazone. Cette région est-elle biogéographiquement homogène au sein de l'Amazonie ? Quelles sont les modalités spatio-temporelles de diversification au sein de cette région ? Afin de répondre à ces questions, j'ai exploré sa biorégionalisation sur la base de la distribution des amphibiens anoures. Cette approche a permis de définir trois biorégions dans l'est du Plateau des Guyanes, et de révéler une forte sous-estimation de l'endémisme. Ensuite, j'ai étudié les patrons de diversification au sein du genre endémique *Anomaloglossus*. Ce volet a permis de dévoiler l'existence de spéciation cryptique au sein du genre, avec un patron biogéographique composé de quatre zones de diversification au sein du Plateau des Guyanes et une origine du genre dans les tepuis.

Mots clés Amazonie. Plateau des Guyanes. Diversité. Biorégionalisation. Endémisme. Diversification. Biogéographie. Évolution. Taxinomie intégrative. Amphibiens.

Discipline Écologie, biodiversité et évolution

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